THE REACTION BETWEEN

FERROCYTOCHROME *c* AND HYDROGEN PEROXIDE

A Thesis

Presented to the University

of Cape Town

for the Degree of Master of Science

by

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The University of Cape Town

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SUMMARY

The reaction between ferrocytochrome c and hydrogen peroxide has been studied to determine possible reaction mechanisms. This was done by investigating the effect of various physical factors, such as hydrogen ion concentration, ionic strength and temperature, on the reaction and by studying the kinetics of the reaction. The study of pH and ionic strength showed that an ionization occurred at pH 7.8 which influenced the reactivity of the cytochrome c. The reaction was studied kinetically at pH 8.1 and pH 6.6. At pH 8.1 simple kinetics showed an uncomplicated reaction between ferrocytochrome c and hydrogen peroxide. The kinetic studies at pH 6.6 showed that ferrocytochrome c, hydrogen peroxide and ferricytochrome c (acting as a product catalyst) were involved in the reaction. The product catalysis complicated the kinetics and difficulty was experienced in fitting a suitable reaction mechanism.

Copper ions have been found to catalyse reactions similar to that studied here. The effect of a copper - histidine complex was therefore investigated on the reaction at pH 8.7. The copper complex was found to catalyse the oxidation of ferrocytochrome c by hydrogen peroxide and the kinetic studies of this oxidation showed that the reaction could be fitted to a simple

mechanism which did not involve the formation of intermediate complexes. An unusual feature of the reaction was the ability of the cytochrome c, after complete oxidation, to undergo reduction, which is dependent on the presence of both hydrogen peroxide and copper ions.

I. INTRODUCTION

A. Cytochrome c

Cytochrome c is a haemoprotein present in all living cells, where it plays an important part in the capture of energy in the mitochondrial electron transport mechanism. It participates in redox reactions as ferricytochrome c and ferrocytochrome c corresponding respectively to the Fe(III) and Fe(II) oxidation states of the haem iron.

The absorption spectrum of cytochrome c was first observed by McMunn,[1] in 1886, in a large variety of organisms and tissues, but this important observation was overlooked for several decades. In 1930, Keilin[2] produced, from Delft yeast, the first purified preparation of cytochrome c. Cytochrome c, unlike other cytochromes and haemoproteins, was found not to bind reagents such as carbon monoxide, nitrogen and cyanide. Keilin and Hartree[3] showed that this inability of the cytochrome c to bind these reagents was due to the binding of the 5 and 6 coordination sites of the haem iron, to the protein molecule. This has been supported by the studies of Theorell and Akesson[4] and more recently Vinogradov and Harbury[5] who have investigated which amino acid residues are most likely involved in

coordination with iron.

Recent crystallographic work by Dickerson etal[6] involving a 4Å resolution X ray study of ferricytochrome c has helped to elucidate several structural features of the protein. As the structure of cytochrome c has been almost completely determined, it should soon be possible to relate the reactivity of the molecule to its structural features, which apparently alter during redox reactions and with changes in pH and temperature.

B. Reactions of Cytochrome c

Cytochrome c participates in a number of redox reactions, both in the presence and in the absence of enzymes, involving such reagents as ascorbic acid, hydroquinone, $NADH_2$, ferricyanide, oxygen and hydrogen peroxide.

(1) Enzyme Catalysed Reactions of Cytochrome c

Cytochrome c is a substrate for several enzymes including cytochrome oxidase, the peroxidases and $NADH_2$: cytochrome c reductase. The most intensively studied of these is cytochrome oxidase which catalyses the reaction

$$4Fe^{2+}cyt\underline{c} + O_2 \longrightarrow 4Fe^{3+}cyt\underline{c} + 2H_2O$$

i.e. the terminal reaction of the mitochondrial electron transport chain. Much work has been done on the extraction and purification of cytochrome oxidase and on

the mechanism of the reaction. Recent reviews by Lemberg[7] and Mason[8] include much of the work done on this enzymic reaction.

Ferrocytochrome c is oxidized by hydrogen peroxide in the presence of horse radish peroxidase or cytochrome c peroxidase. Chance[9], Yonetani[10], Beetlestone[11] and Nicholls[12] have carried out kinetic and spectral studies on the cytochrome c peroxidase system. The results indicate that the enzyme first binds the hydrogen peroxide and then reacts successively with two ferrocytochrome c molecules which provide the necessary electrons for the reduction of the peroxide. The reaction of cytochrome c with the peroxidase-peroxide intermediate is the fastest protein-protein interaction yet measured in solutions, having a second order rate constant of the order of 10^9 l.mole^{-1}sec^{-1}

In none of these enzyme reactions does any direct interaction occur between ferrocytochrome c and the other substrate. Instead cytochrome c acts as an electron donor to the enzyme, which then passes the electron to the bound substrate.

(2) Non Enzymic Reduction of Cytochrome c

Cytochrome c is directly reduced by ascorbate or hydroquinone in the absence of enzymes. The reactions have been studied by Greenwood and Palmer and by Williams.

In their studies of the ascorbate reduction of ferricytochrome c, Greenwood and Palmer[13,14] observed a slow second order reaction, dominant at pH 7 and a reaction independent of cytochrome c concentration, at higher pH values. They have shown a pH dependent equilibrium between these two reactions. They have interpreted these observations in terms of a fundamental change in the tertiary structure of the haemoprotein, dependent on pH, which gives rise to two forms of ferricytochrome c. Skov and Williams[15] have also studied this reaction and, although not disputing any of the findings of Greenwood and Palmer, they suggest that simultaneous changes in the ionic strength might have been the determining influence on the reactivity of cytochrome c in Greenwood's experiments.

Williams[16] has studied the reduction of ferricytochrome c by hydroquinone. He obtained evidence indicating that p-quinone, a product of the reaction, acts as a catalyst for the reduction of cytochrome c. He also found that the effect of the hydrogen ion concentration approximates to a linear relationship between pH 6.4 and pH 7.6, which is consistant with the potentiometric studies of Rodkey and Ball[17] on cytochrome c, which indicate no ionization occurring between pH 1.7 and pH 7.8.

(3) Oxidation of Ferrocytochrome *c* by Oxygen.

The value of the redox potentials for cytochrome *c* shows that the oxidation of the reduced protein by oxygen is thermodynamically favourable. The fact that ferrocytochrome *c* is not autoxidizable, except at extremes of pH must therefore be due to kinetic barriers. Boeri and Tosi[18,19] investigated the kinetics of the reaction in acid and alkaline conditions, showing that the autoxidation rate is proportional to the concentration of ferrocytochrome *c*, the proton activity, the oxygen partial pressure and the ionic strength of the solution. They proposed the rate equation

$$-\frac{d[Fe^{2+}cyt\underline{c}]}{dt} = k[Fe^{2+}cyt\underline{c}][H^+](pO_2)\mu$$

which accounted for these observations.

Boeri and Tosi also observed that autoxidation of ferrocytochrome *c* is substantially accelerated by the presence of trichloroacetate and other halogenated carboxylic acids. Schejter and George[20] have shown that trichloroacetate reversibly alters the conformation of ferricytochrome *c*.

Margoliash and Schejter[21] in an excellent critical review of cytochrome *c* point out the need for considerably more kinetic data than is at present available, for an understanding of the function of cytochrome *c* and its

reactions.

(4) Oxidation of Ferrocytochrome c by Hydrogen Peroxide.

The value of the redox potential for the reaction involving ferrocytochrome c and hydrogen peroxide, given by the equation

$$Fe^{2+}cyt\underline{c} + \tfrac{1}{2}H_2O_2 + H^+ \longrightarrow Fe^{3+}cyt\underline{c} + H_2O$$

is $\Delta E_o = 1.547$ volts. This can be used to calculate the free energy of the reaction at $[H^+] = 1$, which is found to be $\Delta G = -35 kcal.mole^{-1}$, showing that the reaction is thermodynamically favourable.

The reaction between ferrocytochrome c and hydrogen peroxide has been observed during studies of the cytochrome c peroxidase reaction. Beetlestone[11]. Nicholls[12] and Yonetani[10] noted a direct reaction between ferrocytochrome c and hydrogen peroxide when they were mixed prior to the addition of the enzyme, and used concentrations of ferrocytochrome c and hydrogen peroxide that rendered the non-enzymatic oxidation negligible.

O'Brein[22] has studied the products of the action of hydrogen peroxide on ferricytochrome c and observed that between pH 4 and 10 there is a decrease in peak height for the α, β and Soret peaks of the ferricytochrome c spectra. It is thought that this decrease in peak

height may involve a conformational change in the protein, since the cytochrome c, on reduction is more autoxidizable than before.

Davison and Hamilton[23] have performed experiments on the effect of pH on the reaction between ferrocytochrome c and hydrogen peroxide. They found that at suitable hydrogen peroxide concentrations, the reaction occurred throughout a wide range of pH values, although it became slow at pH values above 9. At the lower pH values, the rate curves indicated zero order reations, while at higher pH values, this changed to first order reactions.

Shashoua[24] has performed the only kinetic studies reported for the ferrocytochrome c-hydrogen peroxide reaction using magneto-optical rotation spectroscopy. Working at pH 9 and with a hundredfold excess of hydrogen peroxide, he has observed a first order reaction with respect to ferrocytochrome c. Using rate constants measured for temperatures from 9°C to 21°C in the Arrhenius equation he has calculated an activation energy of 3 - 5 kcal.mole^{-1}. However when the reaction was performed at pH 7 in a 700 fold excess of hydrogen peroxide in phosphate buffer, the reaction followed neither first- nor second-order kinetics. In fact it seemed to approximate a first-order reaction during the

initial 40 - 60% of the reaction followed by a transition period and then a second region of first order kinetics, with a different rate.

He has postulated that the reaction could take place by two consecutive first order reactions in which the ferricytochrome c gives up one electron at a time through an intermediate complex. Another proposal is the formation of an intermediate dimer between one molecule of ferrocytochrome c and one molecule of ferricytochrome c.

Although little is known of the reaction between ferrocytochrome c and hydrogen peroxide, the reaction between ferrous ions and hydrogen peroxide in solution has been comprehensively studied and is reviewed by Baxendale[25] and Weiss[26]. It has been found that when the ferrous ions are in excess the reaction followed the simple mechanism

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$$

$$Fe^{2+} + {}^{\bullet}OH \longrightarrow Fe^{3+} + OH^-$$

However when the hydrogen peroxide is in excess, then oxygen is also evolved from the system and further reaction steps are necessary to explain this reaction. In all the kinetic studies the reaction was found to be bimolecular, following the simple rate equation

$$-\frac{d[Fe^{2+}]}{dt} = -2\frac{d[H_2O_2]}{dt} = 2k_1 [Fe^{2+}] [H_2O_2]$$

The rate constant for this reaction was found to be of the order of 60 l.mole^{-1} sec.$^{-1}$ at 25°C and the activation energy was found to be 10 kcals.mole^{-1} (27). It has been found that ferric ions inhibit the reaction causing deviations from bimolecularity and these deviations are found to increase as the concentration of ferric ions increase, or as the pH increases.

The purpose of the present study was to investigate the mechanism of the reaction between ferrocytochrome c and hydrogen peroxide, in an attempt to account for the zero order reactions observed by Davison and Hamilton(23) and the change in reactivity as the conditions were altered, observed by both Davison and Hamilton(23) and by Shashoua(24). Possible causes of the observed zero order kinetics, including substrate inhibition, activation by intermediates such as free radicals or activation by products of the reaction would all be investigated. Also, as the reaction between ferrous ions and hydrogen peroxide has been thoroughly studied, there exists a basis for determining the effect of the porphyrin-protein portion of the molecule upon the reaction. It was hoped to obtain the required

information by studying the effect of physical factors such as hydrogen ion concentration, ionic strength and temperature on the reaction and also by observing the kinetic behaviour of the system with respect to the reactant concentrations.

Recently interest has been shown in the function of metal ions present in a wide range of proteins and enzymes[28]. Particular interest has been shown in the role of copper in biological redox reactions. This interest has mainly centred around the autoxidation of ascorbic acid by copper ions and copper-amino acid complexes[29,30,31]. Davison etal[32,33] have studied the effect of copper complexes on the autoxidation of ferrocytochrome c and found that the copper catalyses the reaction. It was therefore decided to include an investigation of the influence of a copper-histidine complex on the ferrocytochrome c - hydrogen peroxide reaction in the present study.

II. MATERIALS AND METHODS.

A. Buffers

Buffer solutions were prepared using BDH 'Analar' grade reagents and distilled water which had been further purified by ion exchange. The buffers used were the sodium dihydrogen phosphate - disodium hydrogen phosphate buffer and the Kolthoff phosphate buffer comprising sodium dihydrogen phosphate and sodium tetraborate. Stock solutions of 0.1M NaH_2PO_4 and 0.1M Na_2HPO_4 were prepared for the phosphate buffer. To prepare a solution of pH 8.1, 19ml. of Na_2HPO_4 and 1ml. of NaH_2PO_4 were mixed and dilutes to 100ml. To prepare a solution of pH 6.6, 6.5ml. of Na_2HPO_4 and 13.5ml. of NaH_2PO_4 were mixed and diluted to 100ml. For the Kolthoff phosphate buffer, the same stock solution of 0.1M NaH_2PO_4 was used together with a 0.05M NaB_4O_7 solution. A buffer of pH 8.7 was prepared by mixing together 75ml. of the 0.05M NaB_4O_7 solution and 25ml. of the 0.1M NaH_2PO_4 solution.

B. Hydrogen Peroxide

The BDH 'Analar' grade hydrogen peroxide used was 30% w/v and was found to be 8.75M by titration against potassium permanganate. A stock solution of the hydrogen peroxide was prepared by adding 0.2ml. of the 8.75M hydrogen peroxide to 100ml. of water. Alliquots of this

stock solution were then injected into the reaction cuvettes to initiate the reactions.

C. Cytochrome *c*

Horse heart cytochrome *c*, A grade, supplied by Seravac Laboratories, was 94% pure, on the basis of iron content. A cytochrome *c* solution was subjected to disc electrophoresis, using polyacrilamide gels and employing the method of Reisveld, Lewis and Williams[34] for the separation of basic proteins. The cytochrome *c* migrates as one band on the gel, no separation being obtained. Gel filtration was also used, the cytochrome *c* being passed through a Sephadex G 75 column of 1 metre length. The cytochrome *c* again migrated as one band. Further, a sample which was only 84% pure was used for kinetic experiments and the results compared with those obtained using the sample of higher purity. It was found that under similar conditions, identical reactions were observed. Therefore it was concluded that any impurities present had no adverse influence on the reactivity of the cytochrome *c*.

Various methods of reducing the cytochrome *c* were tried. To 25mgm. of cytochrome *c* dissolved in 2ml. of deionized water, 0.5mgm of sodium dithionite was added, causing rapid reduction. The solution was then eluted

through a Sephadex G 25 gel column to separate off the unwanted products of reduction which the sodium dithionite produces[35]. The reduced cytochrome c produced by this method oxidized spontaneously and the method was not studied further. Sodium borohydride was similarly used, but since the sodium borohydride in solution evolves hydrogen gas, the solution could not be passed through a Sephadex column to remove the products of reduction. These reductions were abandoned in favour of the reduction by hydrogen gas in the presence of palladized, or better platinized, asbestos, which has the disadvantage of being much slower[36].

40mgm of cytochrome c was dissolved in 12ml. deionized water and placed in a reduction vessel together with 70 - 100mgm platinized asbestos. The solution was flushed with nitrogen for 30 minutes, with hydrogen for four hours and with nitrogen for a further 30 minutes. Agitation of the solution was provided by a magnetic stirrer. The solution was finally filtered and stored under nitrogen at $4^{\circ}C$. It appeared to be stable under these conditions for 2 - 3 weeks.

D. Copper - Histidine Complex

A copper histidine complex was prepared in solution by dissolving 50mgm of copper sulphate and 76mgm of histidine monohydrochloride in 10ml. of Kolthoff phosphate

buffer at pH 8.7. The histidine complexed with the copper giving a deep blue solution, and prevented the formation of copper hydroxide at higher pH values. When 0.1ml. of this solution was added to 2.9ml. of reaction mixture at pH 8.7 it did not alter this pH value noticably.

E. Assay Prodecure

All kinetic experiments were performed on a Beckman DU2 spectrophotometer with a 10" recorder attached. Spectral studies were made on a Beckman DB recording spectrophotometer. All reactions were carried out in quartz cuvettes of 1cm. light path, in a final volume of 3.0ml. Unless otherwise stated, all reactions were thermostatted at 25°C.

For each reaction, 0.3ml. of reduced cytochrome c, 2ml. of buffer and sufficient deionized water to bring the volume to 2.9ml., were pipetted into a cuvette. The reaction was initiated by injecting into the cuvette 0.1ml. of the stock hydrogen peroxide solution and rapidly mixing with a polythene plunger. In cases where the volume of one of the reactants was varied during a series of experiments, the amount of deionized water added was also adjusted to compensate for this. The progress of the oxidation of ferrocytochrome c was followed by measuring the absorbance (A) at 550mμ. The

absorbancy indices of ferrocytochrome c and ferricytochrome c were assumed to be 27.6 x $10^3 M^{-1} cm^2$ and 9.0 x $10^3 M^{-1} cm^2$ respectively, as given by Margoliash and Frohwirt[37].

III. RESULTS

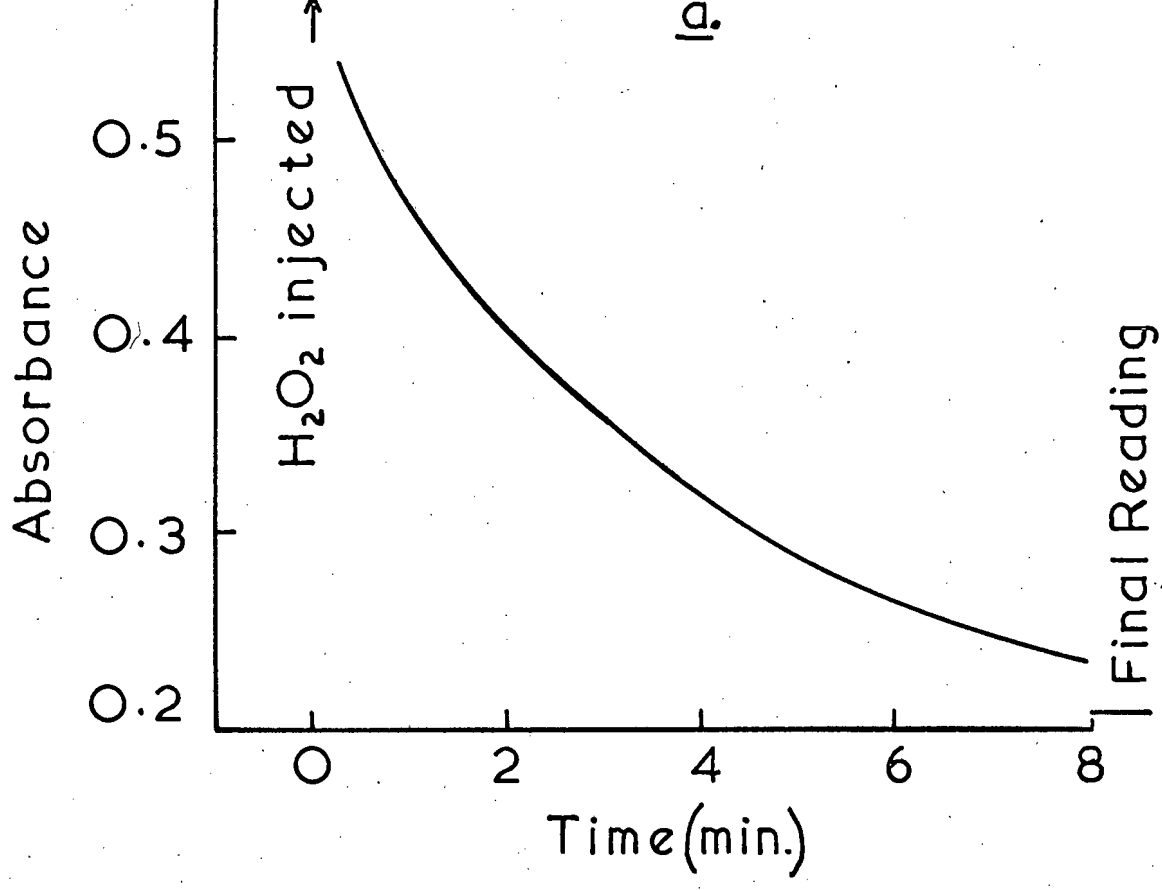
The reaction curves obtained from the experiments were found to be either first order or pseudo zero order, under the experimental conditions employed (see fig. 1 and fig. 2). The initial velocities were obtained in the case of the first order reactions by extrapolating the curves to zero time and measuring the slope of the curve at this point. Rate constants for the first order reactions were obtained by plotting log $(A - A_\infty)$ against time where A_∞ is the absorbance at infinite time. The slope of this plot is equal to $- k/2.303$, where k is the experimental rate constant. In the case of reaction curves which were roughly zero order, the maximum velocity, which is greater than the initial velocity, was used. The apparent zero order rate constants for these reactions were estimated by dividing the maximum velocity by the initial concentration of ferrocytochrome c. Since the reaction curve is linear over the region where maximum velocity is measured, it was considered, as an approximation, to be zero order. This gave the rate equation,

$$v = k[Fe^{2+}cyt\underline{c}_o]$$

where $[Fe^{2+}cyt\underline{c}_o]$ represents the initial concentration and v is the velocity. On rearranging this equation, it

g. 1. a. Typical reaction curve showing trace of Absorbance at 550mμ against time, for reaction in phosphate buffer at pH 8.1.

b. Plot of log $(A-A_\infty)$ against time to determine whether reaction at pH 8.1, with excess hydrogen peroxide is first order.


a.
Absorbance
0.5
0.4
0.3
0.2
H_2O_2 injected
Final Reading
0
2
4
6
8
Time (min.)

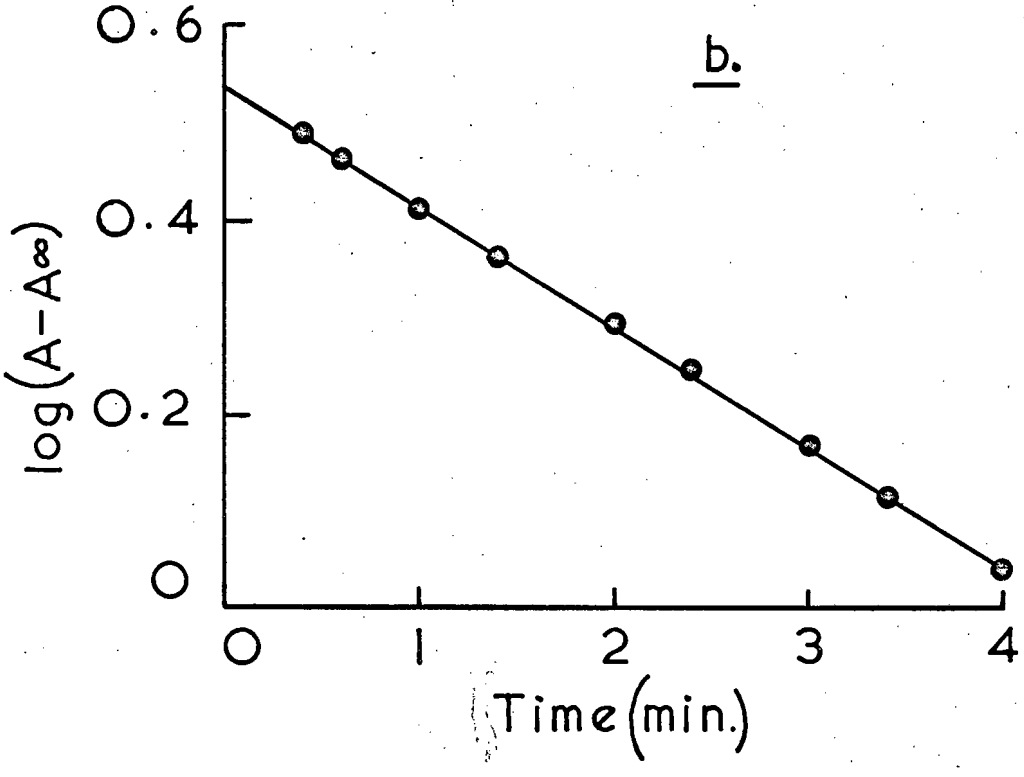
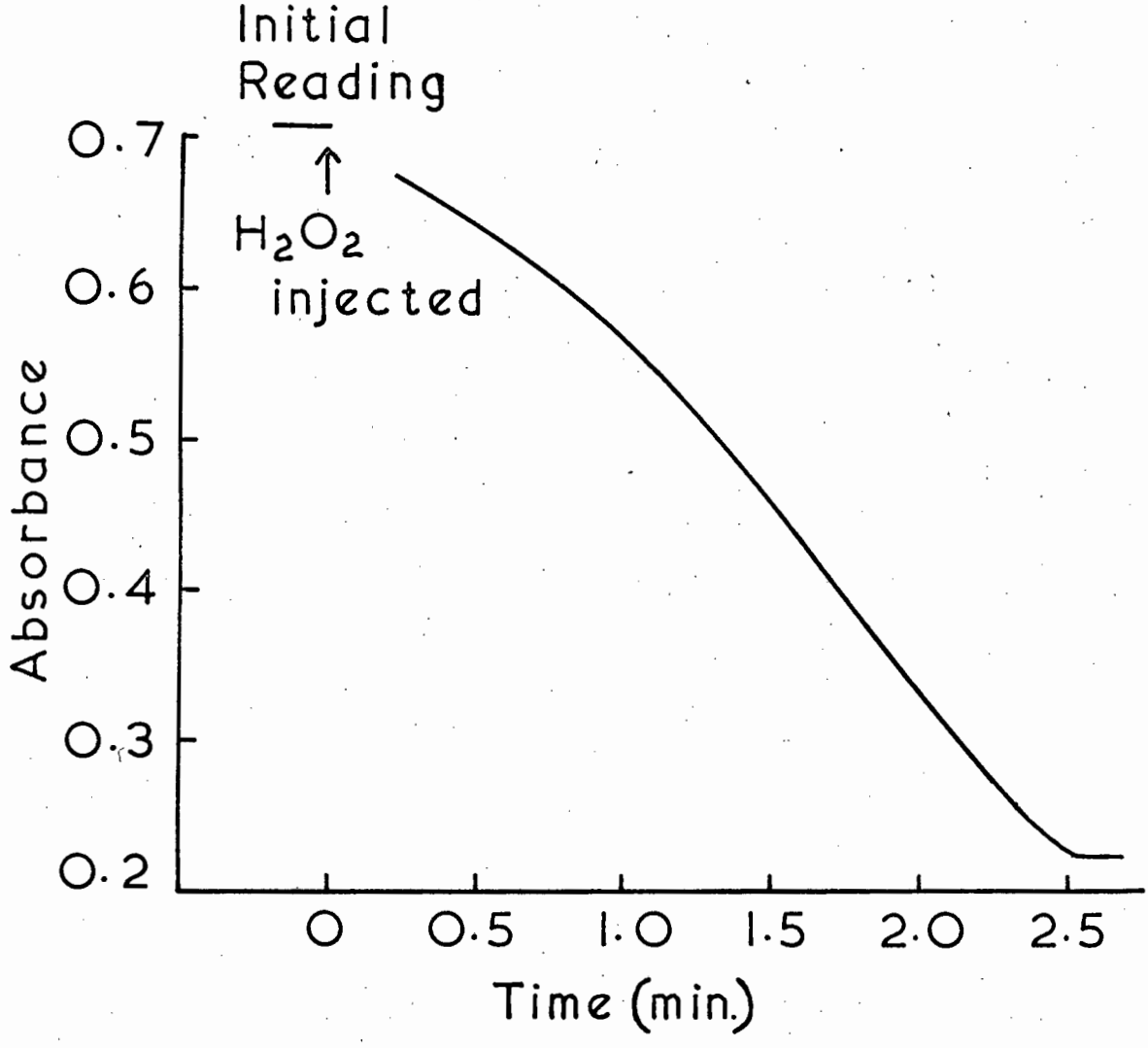

b.
log (A−A∞)
0.6
0.4
0.2
0
0
1
2
3
4
Time (min.)

Fig. 2. Typical reaction curve showing trace of Absorbance at 550mμ against time, for the reaction in phosphate buffer at pH 6.6.


Initial
Reading
H_2O_2
injected
0.7
0.6
0.5
0.4
0.3
0.2
Absorbance
0
0.5
1.0
1.5
2.0
2.5
Time (min.)

gives

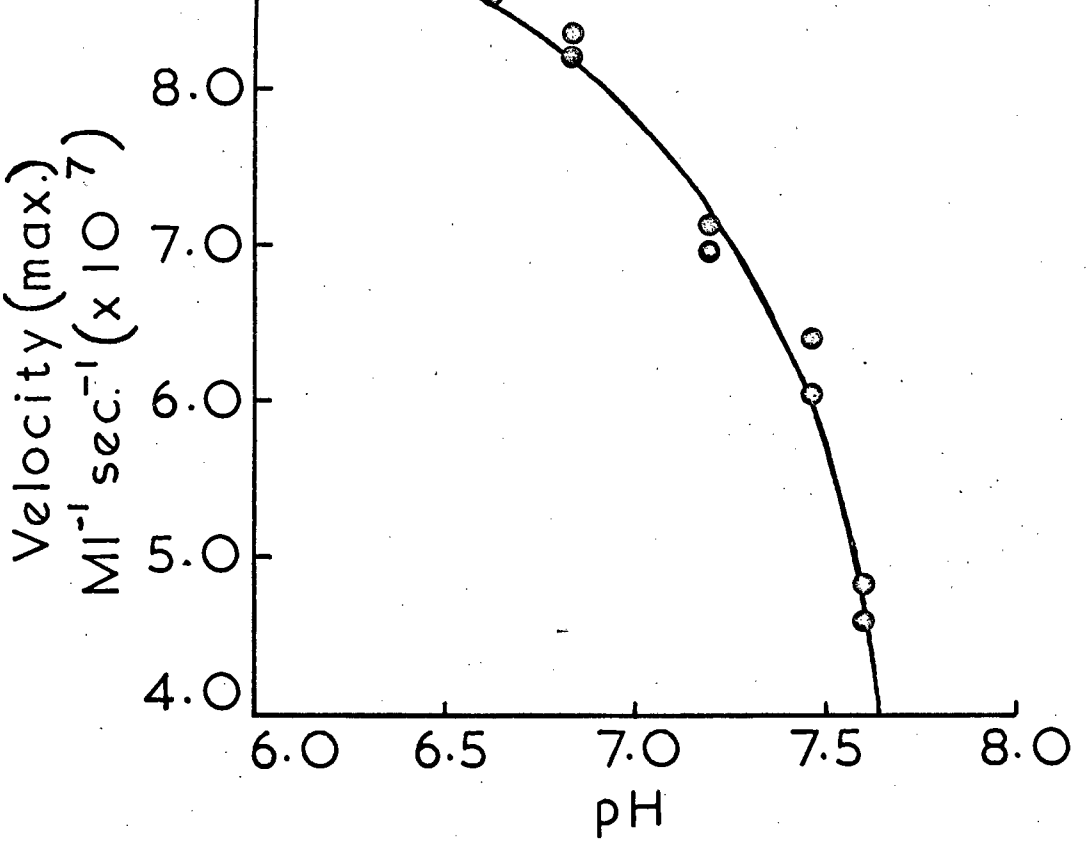
$$k = \frac{v}{[Fe^{2+}cyt\underline{c}_o]}$$

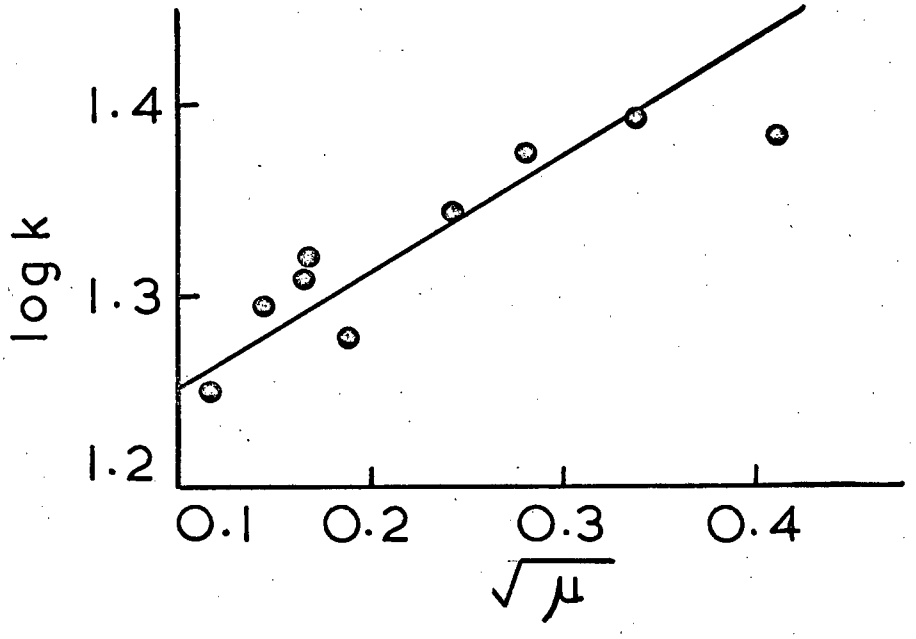
indicating that the rate constant and maximum velocity of the reaction are interrelated.

A. Effect of Hydrogen Ion Concentration and Ionic Strength

The effect of pH and of ionic strength was studied so that the conditions for the reaction between ferrocytochrome c and hydrogen peroxides could be standardized. Phosphate buffer was used in the pH study and in most subsequent studies as the reaction appeared to be well behaved in this buffer. The hydrogen ion concentration was varied between pH 6 and pH 8 and the results are shown in fig. 3. The influence of the hydrogen ion concentration is of interest between pH 7.6 and pH 7.9 where the reaction between ferrocytochrome c and hydrogen peroxide undergoes a significant change. Above pH 7.9 the reaction curves are first order. Below pH 7.6 the reaction curves are approximately zero order after an initial induction period.

The plot of velocity against pH indicates that the reaction slows down as the pH increases to pH 7.6. Above pH 7.9 two reactions were obtained using Kolthoff phosphate buffer.


Velocity (max.)
Ml⁻¹ sec.⁻¹ (x 10⁷)
8.0
7.0
6.0
5.0
4.0
6.0
6.5
7.0
7.5
8.0
pH


log k
1.4
1.3
1.2
0.1
0.2
0.3
0.4
√μ

pH	Velocity
8.00	$4.6 \times 10^{-8} Ml.^{-1} sec.^{-1}$
8.64	$1.4 \times 10^{-8} Ml.^{-1} sec.^{-1}$

This indicates that the rate decreases as the pH increases above pH 7.9, which was also observed by Davison and Hamilton[23].

The effect of ionic strength on the ferrocytochrome c - hydrogen peroxide reaction was studied at pH 6.6 using phosphate buffer. The results of this study are shown in fig. 4 where the logrithm of the rate constant k is plotted against the square root of the ionic strength μ. The plot shows that the rate of reaction increases as the ionic strength of the solution increases. The plot of log k against $\sqrt{\mu}$, from the equation relating the rate of reaction to the ionic strength,

$$\log k = \log k_o + 1.018 z_A z_B \mu^{\frac{1}{2}}$$

which is derived from the Debye Huckel expression for the mean ionic activity coefficients. The slope of the plot is + 0.54 which is equal to the net ionic charge $z_A z_B$ created during the formation of the transition state.

From the results of the pH study it was decided to study the kinetics of the reaction at pH 8.1 and at pH 6.6. The ionic strengths of the phosphate buffers were fixed at $\mu = 0.07$ at pH 8.1 and $\mu = 0.03$ at pH 6.6.

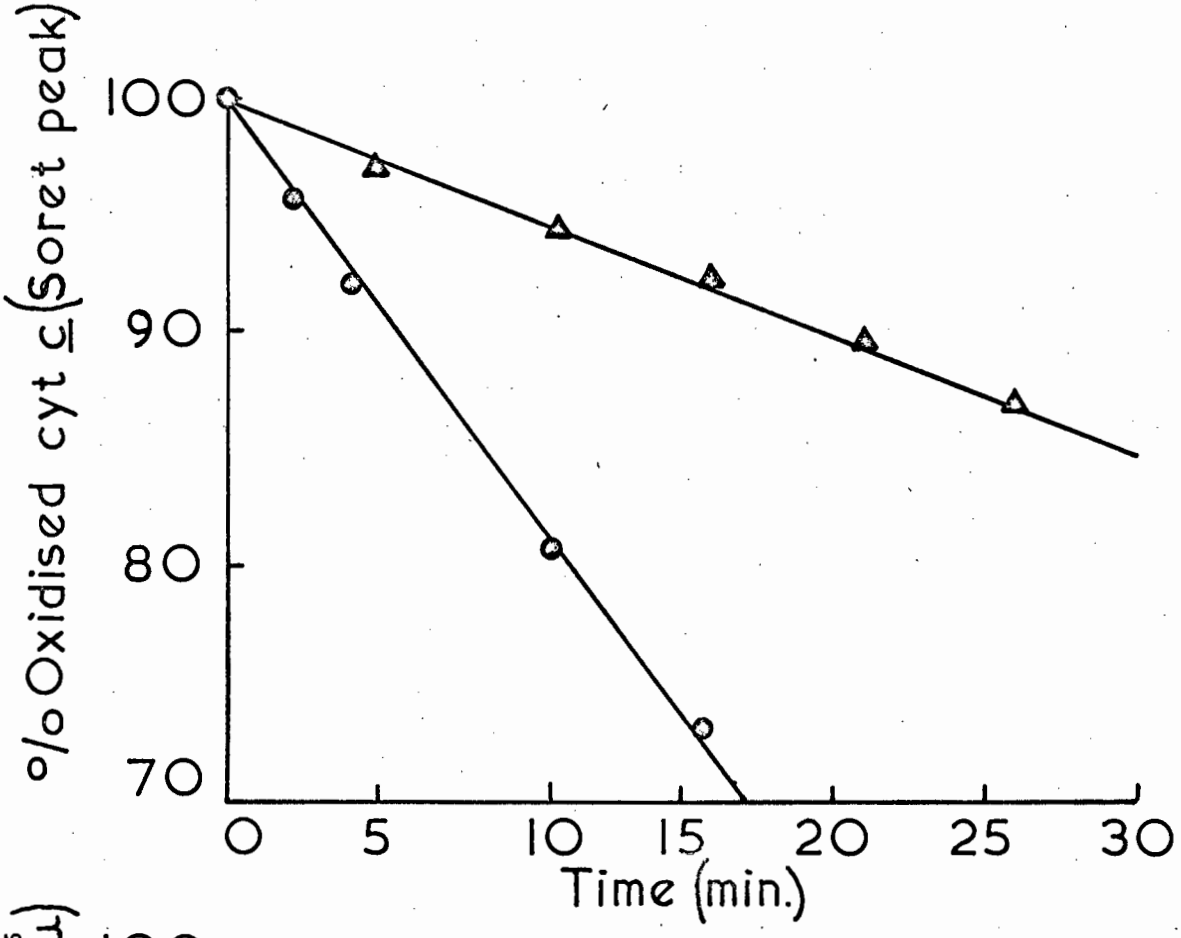
B. Effect of Hydrogen Peroxide on Ferricytochrome *c*

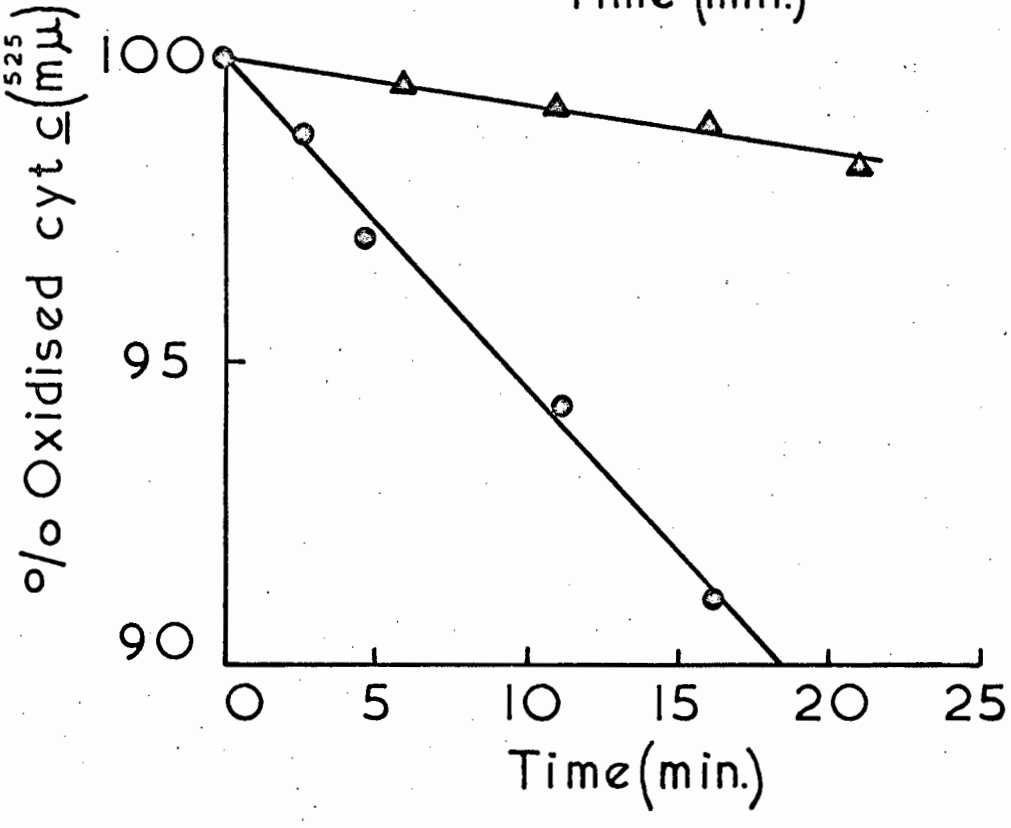
Hydrogen peroxide has been found to act upon ferricytochrome *c*[22]. It causes a decrease in the heights of the α, β and Soret peaks of the absorption spectrum and also causes a loss of activity, indicated by the decreased ability of ferricytochrome *c* to undergo reduction in the presence of $NADH_2$: cytochrome *c* reductase.

Observations of the α and β peaks were therefore made after treating 30μM ferricytochrome *c* with $5.85 \times 10^{-4} M l^{-1}$ hydrogen peroxide at both pH 6.6 and pH 8.1, in phosphate buffer. Successive scans were carried out on a Beckman DB spectrophotometer over the wavelength range 560mμ to 510mμ. The observations of the Soret peak were similarly performed, using 5μM ferricytochrome *c*, and scanning the wavelength range 420mμ to 400mμ. The peak height at time t was expressed as a percentage of the peak height before addition of the hydrogen peroxide and plotted against time in fig.5. From these plots it can be seen that the decrease in peak height is much greater at pH 6.6 than at pH 8.1. Also the decrease in the height of the Soret peak is much greater than that of the α and β peaks.

The rate of modification of the cytochrome *c* is negligible compared to the rate of the ferrocytochrome *c*

Fig. 5. The percentage oxidized cytochrome c present plotted against time, to show effect of hydrogen peroxide on ferricytochrome c. Peak heights were measured for the Soret peak and at 525mμ (▲ indicates measurements at pH 8.1, ● indicates measurements at pH 6.6).


% Oxidised cyt c (Soret peak)
100
90
80
70
0
5
10
15
20
25
30
Time (min.)


% Oxidised cyt c (525 mμ)
100
95
90
0
5
10
15
20
25
Time (min.)

- hydrogen peroxide reaction studied at pH 8.1 and although the modified cytochrome c is produced faster at pH 6.6, the kinetic studies performed at this pH value all involve reactions which reach completion in less than 5 minutes during which time the production of modified cytochrome c is less than 8%. measured at the Soret peak.

The modification is irreversible since the modified cytochrome c, when reduced using sodium dithionite, produced α, β and Soret peaks lower than those obtained before treatment with hydrogen peroxide. The α peak (550mμ) was found on reduction to be 85% of the original height at pH 6.6 and 89% at pH 8.1. The Soret peak was found to be 69% of the original height at pH 6.6 and 91% at pH 8.1, on reduction with sodium dithionite.

C. The Reaction between Ferrocytochrome c and Hydrogen Peroxide at pH 8.1

It has already been seen in the pH studies that above pH 7.9 the reaction is first order when hydrogen peroxide is in excess. A detailed kinetic study of the reaction at pH 8.1 was performed in order to obtain information about reaction mechanisms.

The order of the reaction with respect to ferrocytochrome c and hydrogen peroxide was independently determined. Initial velocities of the reactions were

used in calculations to avoid possible product effects. In determining the order with respect to ferrocytochrome c, the hydrogen peroxide concentration was kept constant at 5.85×10^{-4}M and the ferrocytochrome c was varied between 8μM and 33μM. Changes in the hydrogen peroxide concentration were therefore less than 3% and were thus neglected. Fig.6 shows a plot of log (Initial Velocity) against log [Fe^{2+}cytc]. The slope of this plot gives the order of the reaction, which is found to be one with respect to ferrocytochrome c. This is in agreement with plots of $\log(A-A_\infty)$ against time, which gave straight lines (fig. 1). Values of the experimental rate constant k were obtained from the slopes of these plots and these were plotted against the initial ferrocytochrome c concentrations of the reactions (see fig.7). Straight lines were obtained from these plots, having small positive slopes, although they could be regarded as having zero slope within experimental error. One plot, however, did have a positive slope which could not be regarded as zero. The relationship between the rate of reaction and the ferrocytochrome c concentration has been regarded as simple, although the occasional deviation from zero slope in fig. 7 does suggest that the relationship may be more complicated.

Difficulty was experienced in determining the

Fig. 6. Plot of log (Initial Velocity) against the log (Ferrocytochrome *c* concentration) for the reaction in phosphate buffer at pH 8.1.

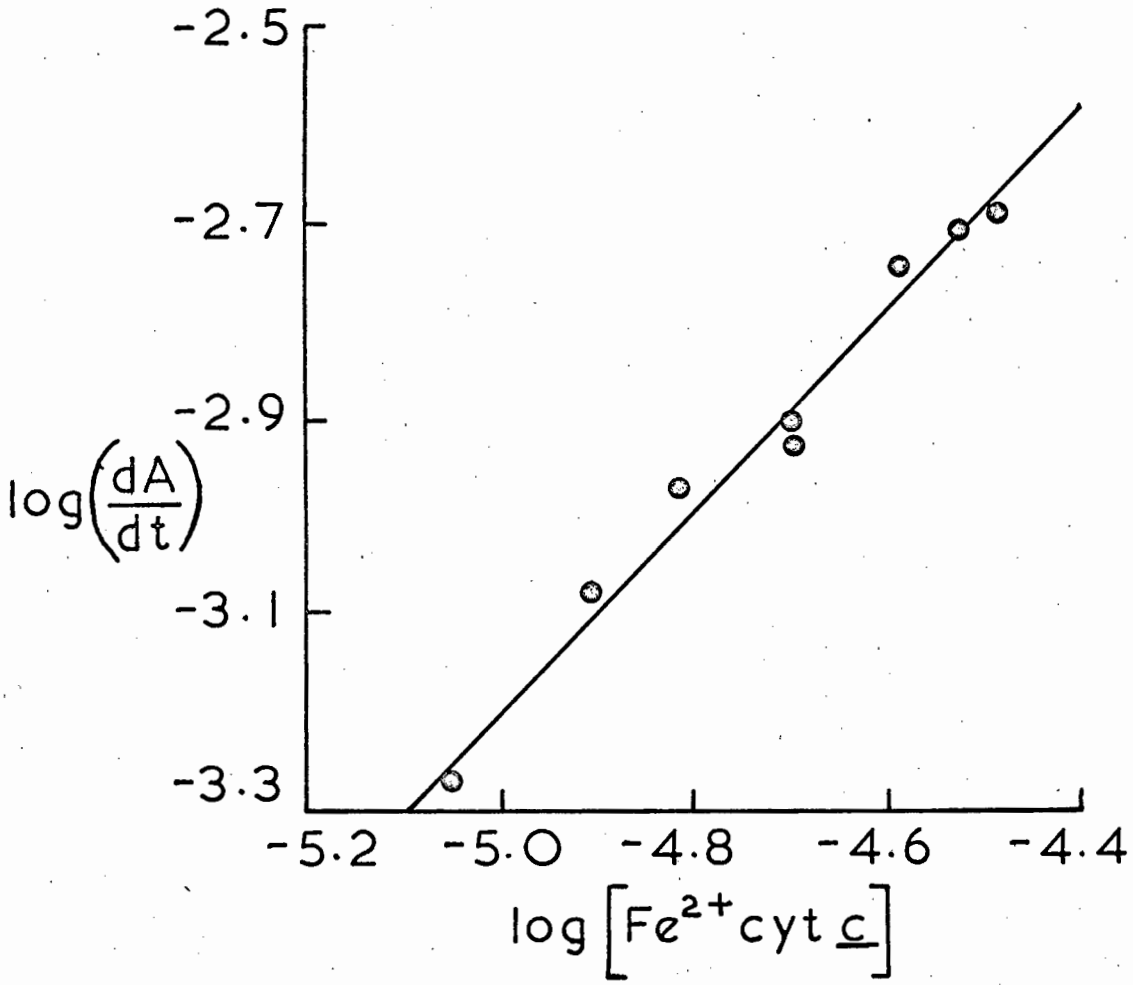
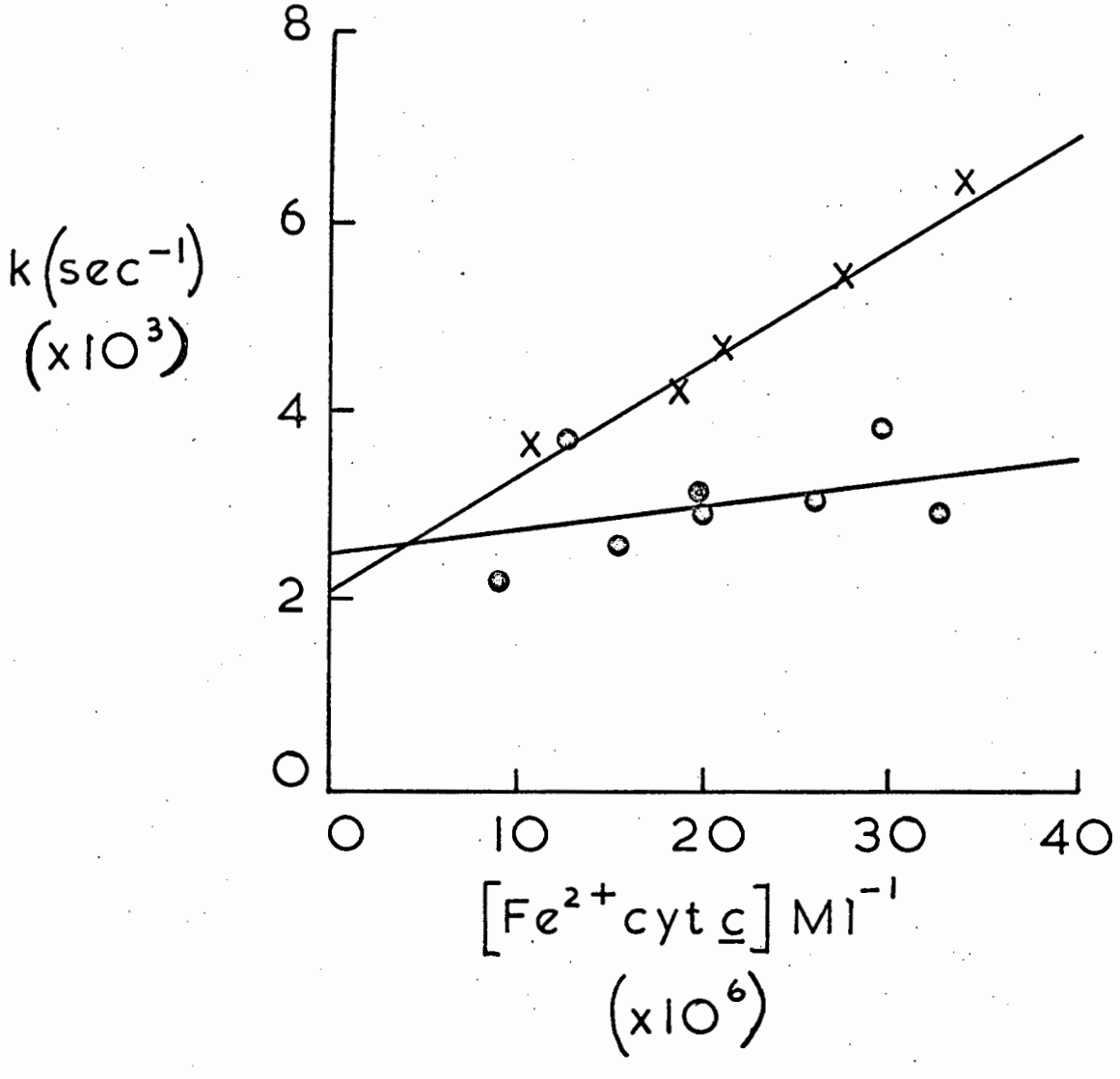

-2.5
-2.7
-2.9
-3.1
-3.3
log(dA/dt)
-5.2
-5.0
-4.8
-4.6
-4.4
log [Fe2+ cyt c]

Fig. 7. Plots of the Rate Constant against Ferrocytochrome c concentration for the reaction in phosphate buffer at pH 8.1.


8
6
4
2
0
k (sec⁻¹)
(x10³)
0
10
20
30
40
[Fe²⁺ cyt c] M1⁻¹
(x10⁶)

influence of hydrogen peroxide on the reaction since ferrocytochrome c has an absorbance greater than 2 at concentrations greater than 70μM. The hydrogen peroxide concentration has to be small in comparison and consequently the absorption changes during a reaction are small and the reaction is slow. Reactions were performed in which the concentrations of both reagents were such that both should influence the order of reaction. 53μM ferrocytochrome c and 23μM hydrogen peroxide were used and the reaction followed on a Beckman DB spectrophotometer. Plots of log $(A-A_\infty)$ and of $\frac{1}{A-A_\infty}$ against time showed that a better fit was obtained for a second order reaction, (see fig. 8) but this result was unreliable since the fit of the first order plot was also reasonable.

Even when in excess of the cytochrome c concentration, the hydrogen peroxide concentration still influences the rate of reaction. Therefore the dependence on hydrogen peroxide was found by varying its concentration, while keeping it in excess of the ferrocytochrome c concentration. The ferrocytochrome c concentration was kept constant at 23μM and the hydrogen peroxide concentration was varied between 1×10^{-4}M and 6×10^{-4}M. The results are shown in fig. 9 where a plot of log (Initial Velocity) against log $[H_2O_2]$ indicated a first

Fig. 8. Plots of log $(A-A_\infty)$ and $\frac{1}{A-A_\infty}$ against time to determine whether reaction between 53 M ferrocytochrome c and 23 M hydrogen peroxide, in phosphate buffer at pH 8.1, is first- or second-order. (▲ indicates plot of log $A-A_\infty$ against time, ● indicates plot of $\frac{1}{A-A_\infty}$ against time.)

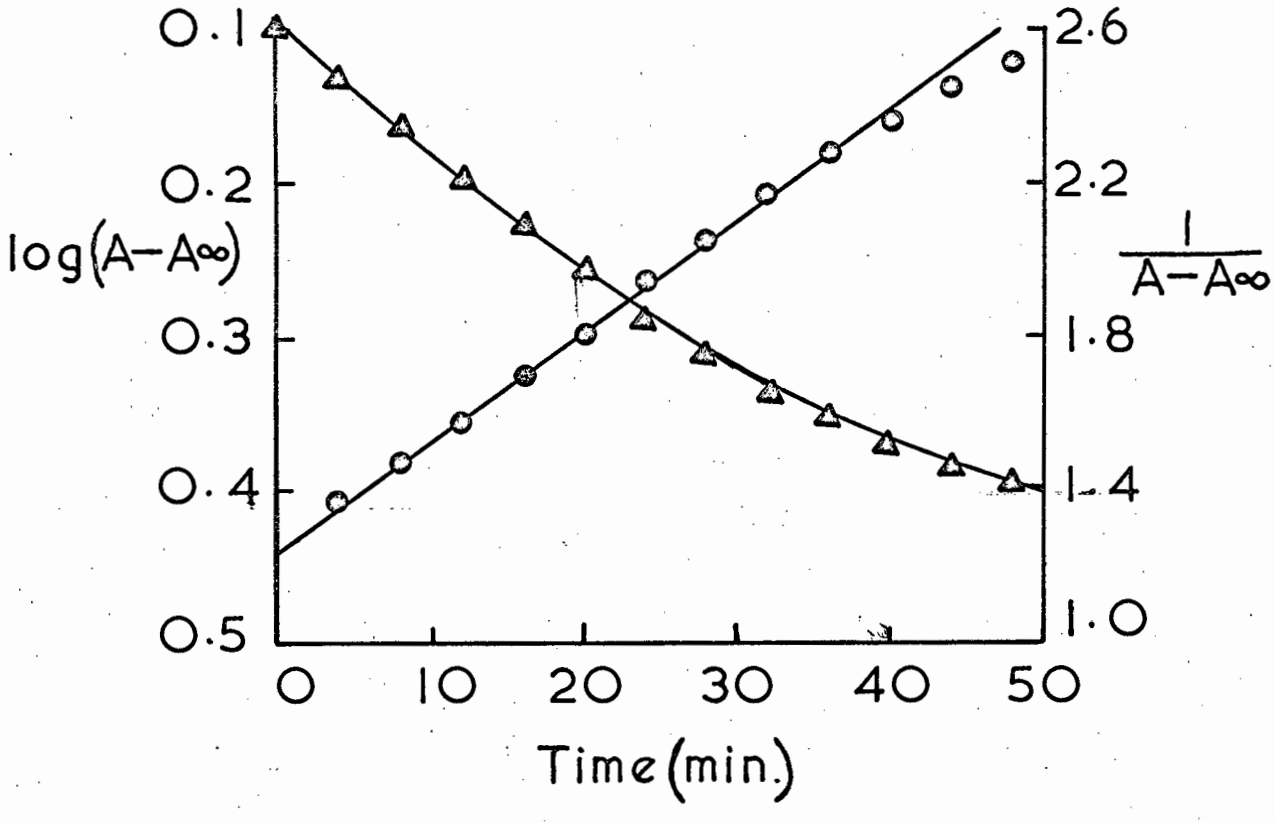
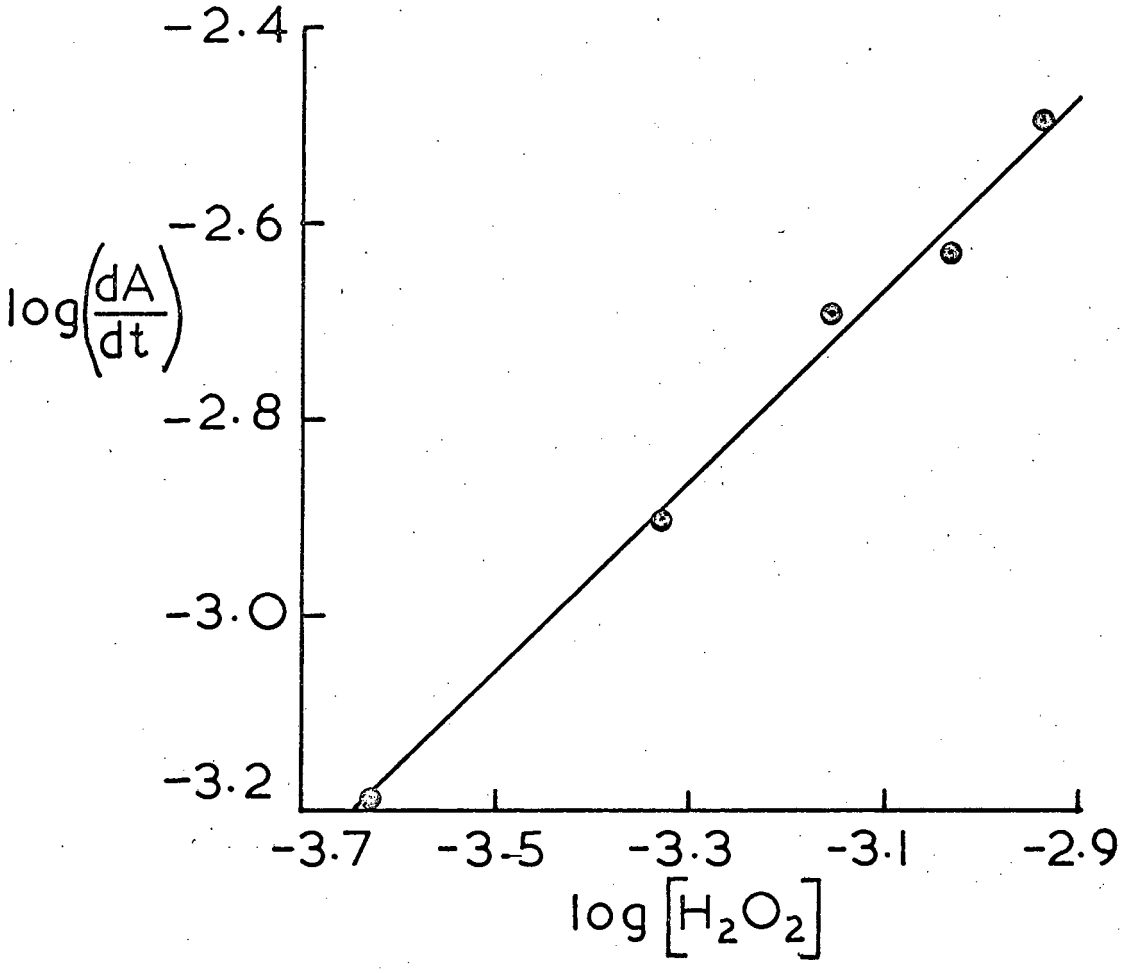

0·1
0·2
0·3
0·4
0·5
log(A−A∞)
2·6
2·2
1·8
1·4
1·0
1/(A−A∞)
0
10
20
30
40
50
Time (min.)

Fig. 9. Plot of log (Initial Velocity) against log (Hydrogen Peroxide concentration) for reaction in phosphate buffer at pH 8.1.


-2.4
-2.6
-2.8
-3.0
-3.2
log(dA/dt)
-3.7
-3.5
-3.3
-3.1
-2.9
log [H2O2]

order dependence on hydrogen peroxide.

When the experimental rate constant k was plotted against hydrogen peroxide concentration, a straight line was obtained which passed close to the origin (see fig.10). A simple dependence of the rate of reaction on the hydrogen peroxide concentration was therefore assumed.

Reactions were also performed to see if ferricytochrome c, a product of the reaction, had any influence upon the reaction. Both ferrocytochrome c and hydrogen peroxide initial concentrations were kept constant and small amounts of ferricytochrome c were added. The results are summarized in Table 1.

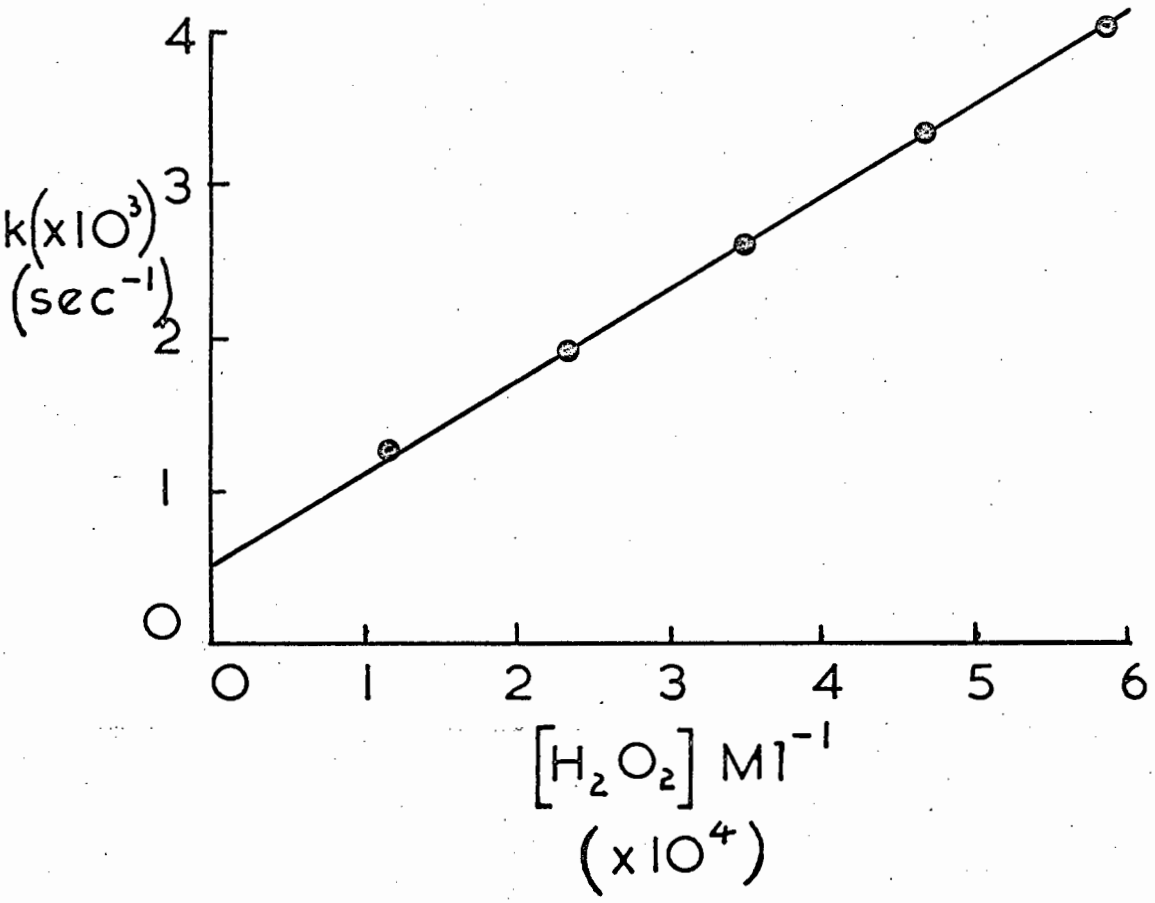
Table 1. Rate constants for varying amounts of ferricytochrome c added to reaction at pH 8.1

Fe^{2+}cyt c	Fe^{3+}cyt c	Exptl. Rate Const.k
16.0μM	1.0μM	2.14×10^{-3} sec.$^{-1}$
16.0μM	4.8μM	1.96×10^{-3} sec.$^{-1}$
20.0μM	1.2μM	2.78×10^{-3} sec.$^{-1}$
20.0μM	2.8μM	3.22×10^{-3} sec.$^{-1}$
20.0μM	2.3μM	3.03×10^{-3} sec.$^{-1}$

From the table it is observed that within experimental error the rate does not alter with added product.

These results have shown that the rate constant for the reaction is proportional to both ferrocytochrome c and hydrogen peroxide and is not influenced by the

Fig. 10. Plot of the Rate Constant against Hydrogen Peroxide concentration for reaction in phosphate buffer at pH 8.1.


4
3
2
1
0
k(x10³)
(sec⁻¹)
0
1
2
3
4
5
6
[H₂O₂] M1⁻¹
(x10⁴)

products of the reaction. For reactions where the ferrocytochrome c dependence was studied, a first order rate constant of 3.0×10^{-3} sec.$^{-1}$ was obtained from fig. 7. This rate constant does not allow for the hydrogen peroxide dependence as the hydrogen peroxide was in excess. By dividing the first order rate constant by the hydrogen peroxide concentration, this dependence is allowed for and a second order rate constant is obtained of 5.1 l.mole^{-1}sec.$^{-1}$. Where the hydrogen peroxide dependence studied, the slope of the plot of k against $[H_2O_2]$ (see fig.10) gave the second order rate constant of 5.9 l.mole^{-1}sec.$^{-1}$. The rate constant for the reaction between ferrocytochrome c and hydrogen peroxide at pH 8.1 was therefore found to be of the order of 5 l.mole^{-1}sec.$^{-1}$.

D. Reaction between Ferrocytochrome c and Hydrogen Peroxide at pH 6.6

The pseudo zero order reactions observed below pH 7.6 in the study of the effect of hydrogen ion concentration, were studied in more detail at pH 6.6 in an attempt to determine the hypothetical reaction mechanism.

The order of reaction was found separately for ferrocytochrome c and hydrogen peroxide. The same technique was used for determining the dependence of

the rate on hydrogen peroxide as was used in the kinetic studies at pH 8.1. The initial ferrocytochrome c concentration was kept constant at 23μM and the hydrogen peroxide concentration was varied between 1 x 10^{-4}M and 6 x 10^{-4}M. The results are represented in fig.11 by the plot of log (Max. Velocity) against log $[H_2O_2]$, which had a slope of unity, the order of the reaction with respect to hydrogen peroxide. The experimental rate constant k, calculated from the maximum velocities, was plotted against the initial hydrogen peroxide concentration (see fig. 12). This plot gave a straight line, which passed through the origin indicating a simple first order dependence of the rate on the hydrogen peroxide concentration.

The influence of ferrocytochrome c on the rate was determined using a constant initial concentration of 5.85 x 10^{-4}M hydrogen peroxide. This ensured that the hydrogen peroxide concentration did not significantly alter during a reaction and could be regarded as a constant. The initial ferrocytochrome c concentration was varied between 5μM and 32μM. The results of these experiments are shown in the plot of log (Max. Velocity) against log [Fe^{2+}cyt c] (see fig.13), where the slope of the plot was found to be unity. This indicates a first order dependence of the rate on the ferrocytochrome c.

Fig. 11. Plot of log (Maximum Velocity) against log (Hydrogen Peroxide concentration) for the reaction in phosphate buffer at pH 6.6.

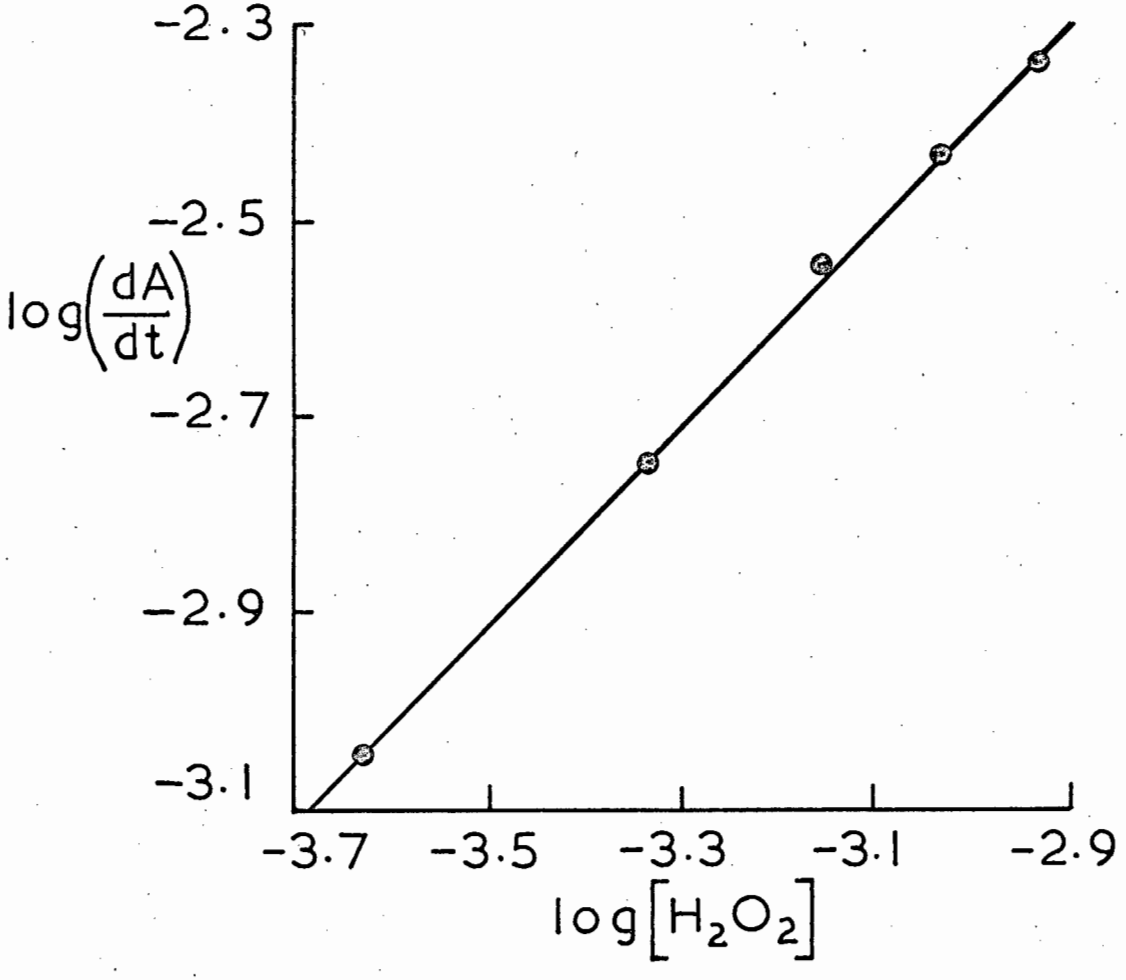

-2.3
-2.5
-2.7
-2.9
-3.1
log(dA/dt)
-3.7
-3.5
-3.3
-3.1
-2.9
log[H2O2]

Fig. 14. Plot of the Rate Constant against Ferrocytochrome c concentration for the reaction in phosphate buffer at pH 6.6

g. 12. Plot of the Rate Constant against the Hydrogen Peroxide concentration for the reaction in phosphate buffer at pH 6.6.

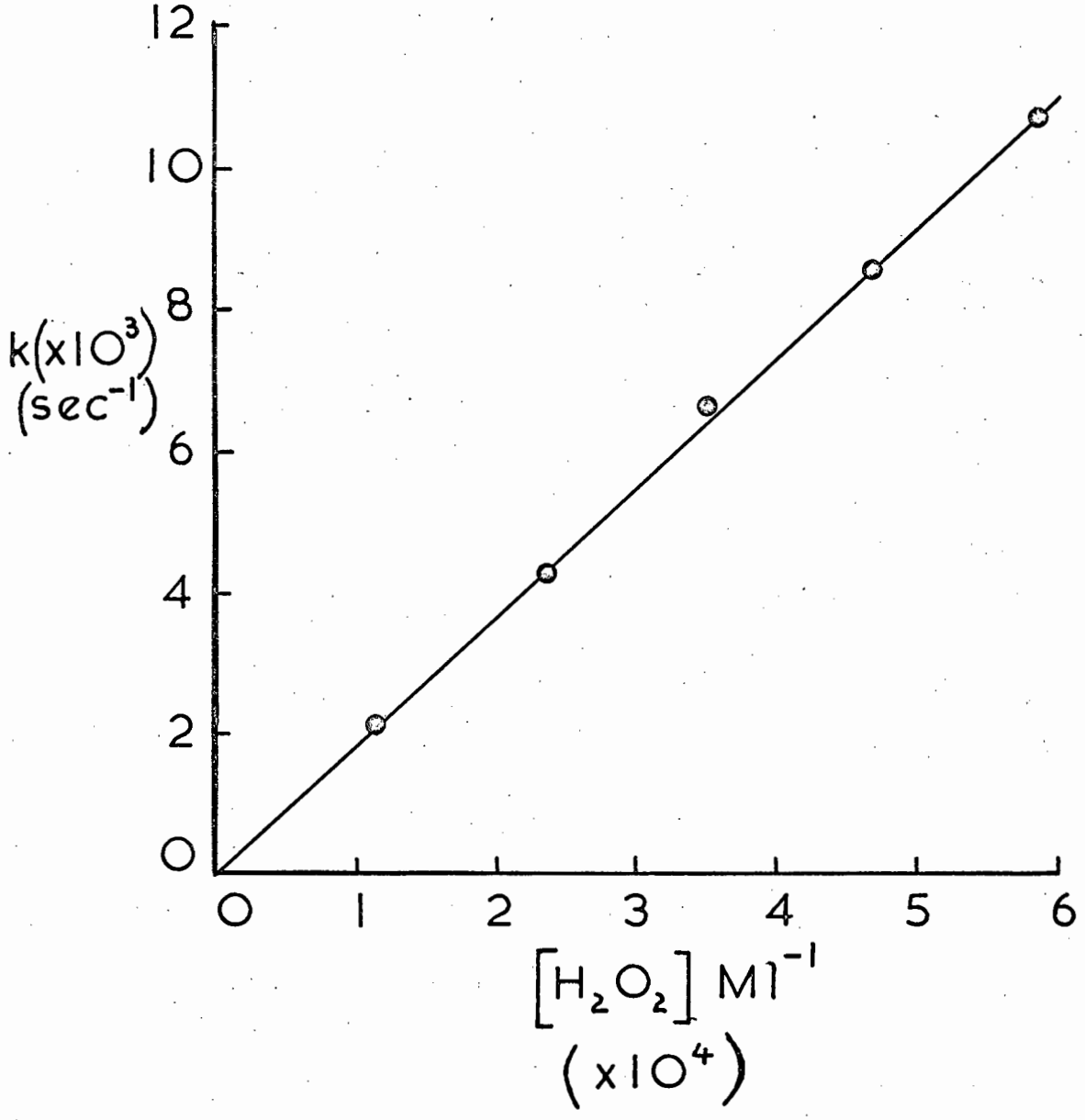
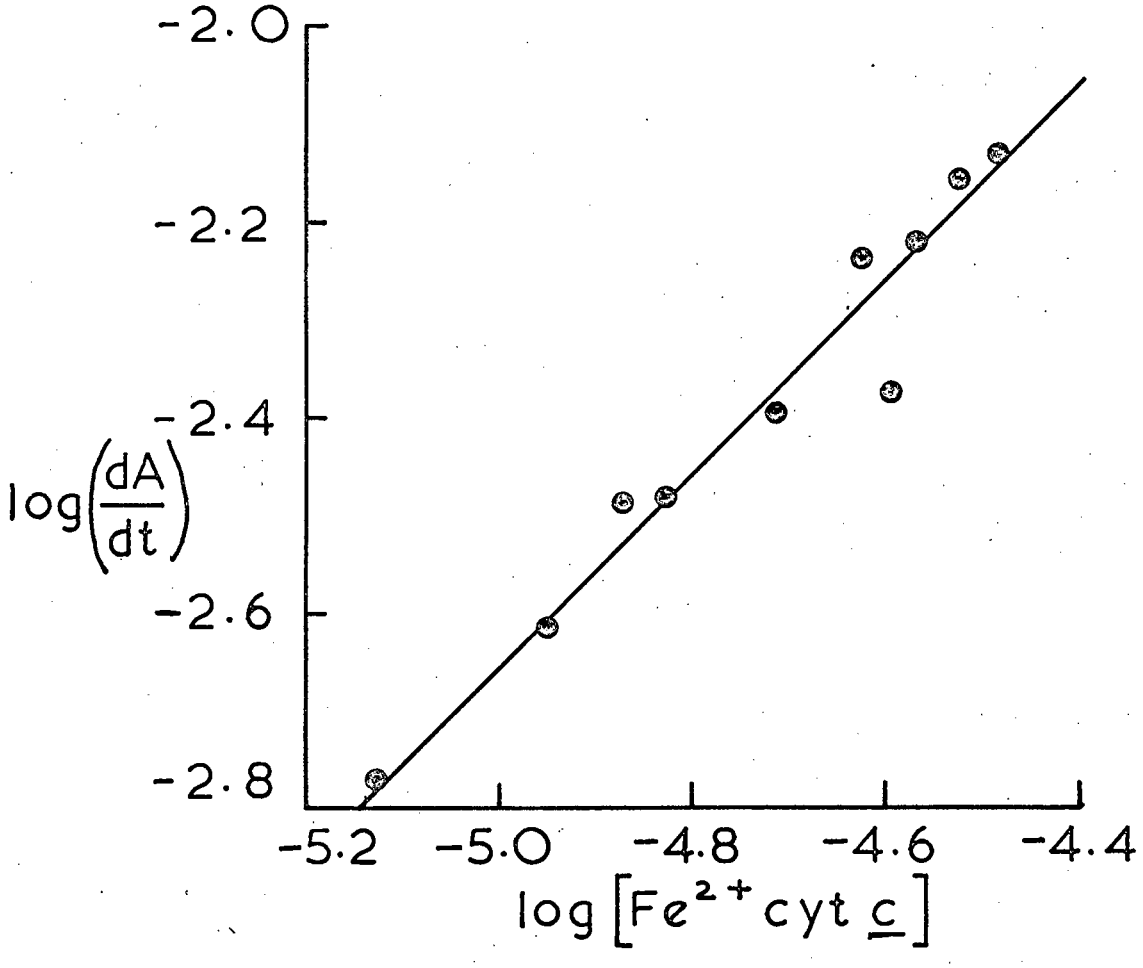

12
10
8
6
4
2
0
k(x10³) (sec⁻¹)
0
1
2
3
4
5
6
[H₂O₂] M l⁻¹ (x10⁴)

Fig. 13. Plot of log (Maximum Velocity) against log (Ferrocytochrome c concentration) for the reaction in phosphate buffer at pH 6.6.


-2.0
-2.2
-2.4
-2.6
-2.8
log(dA/dt)
-5.2
-5.0
-4.8
-4.6
-4.4
log [Fe2+ cyt c]

A plot of the experimental rate constant k against [Fe^{2+}cyt*c*] gave a straight line passing through the origin of the graph. (see fig.14). These results for the dependence of rate on ferrocytochrome *c* suggests the existence of a simple relationship. However this does not account for the pseudo-zero order reaction curves which are obtained experimentally. Laidler[38] has shown that where the order of a reaction with respect to concentration (that is the 'true order'[39]) differs from the order determined from a single reaction (that is the order with respect to time), then this difference is due to the influence of products on the reaction. In the case where the 'true' order is greater than the order with respect to time, the observed effect is product activation of the reaction.

The influence of ferricytochrome *c* on the reaction was therefore investigated. The initial concentrations of both ferrocytochrome *c* and hydrogen peroxide were kept constant at 17μM and 5.85×10^{-4}M respectively and amounts of ferricytochrome *c*, varying between 1μM and 16μM, were added. The results of these reactions are shown in fig.15 where a plot of maximum velocity against [Fe^{3+}cyt*c*] shows that the reaction is product activated. A plot of log (Max. Velocity) against log [Fe^{3+}cyt*c*] (see fig.16) gave a straight line with a slope of 0.46 which was taken

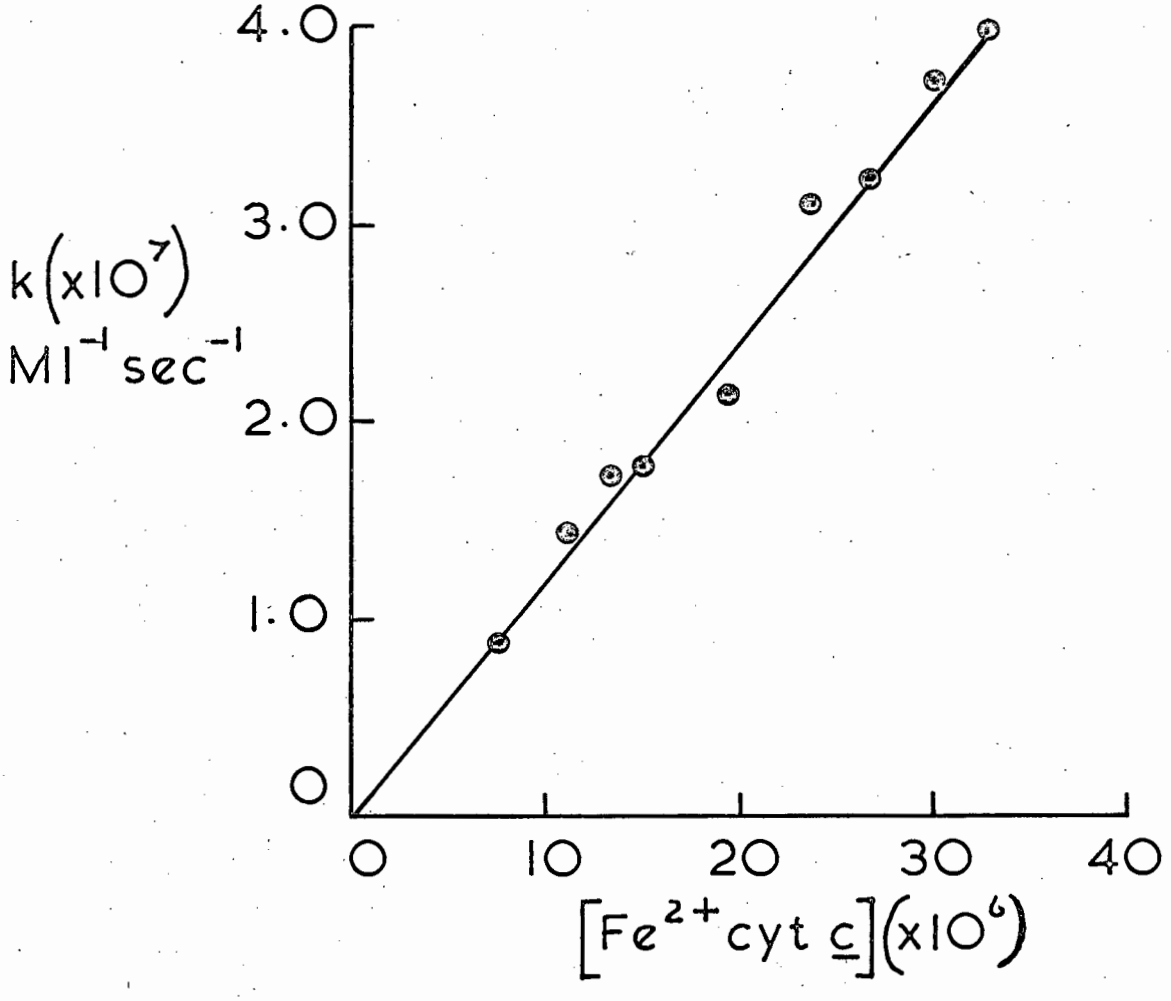
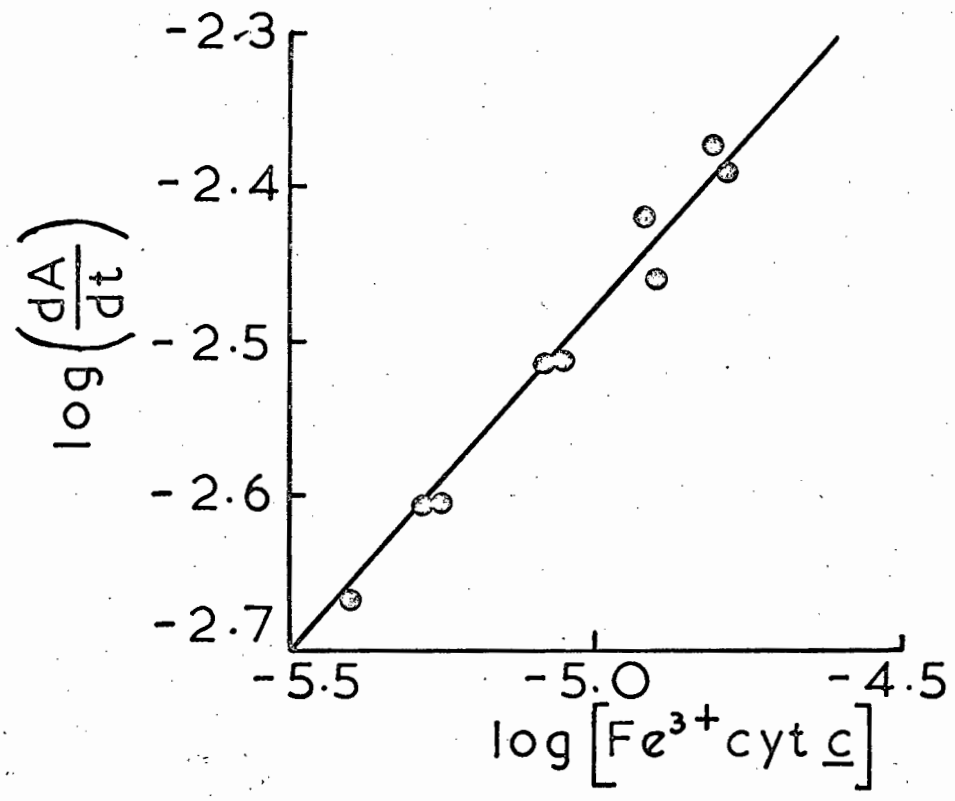
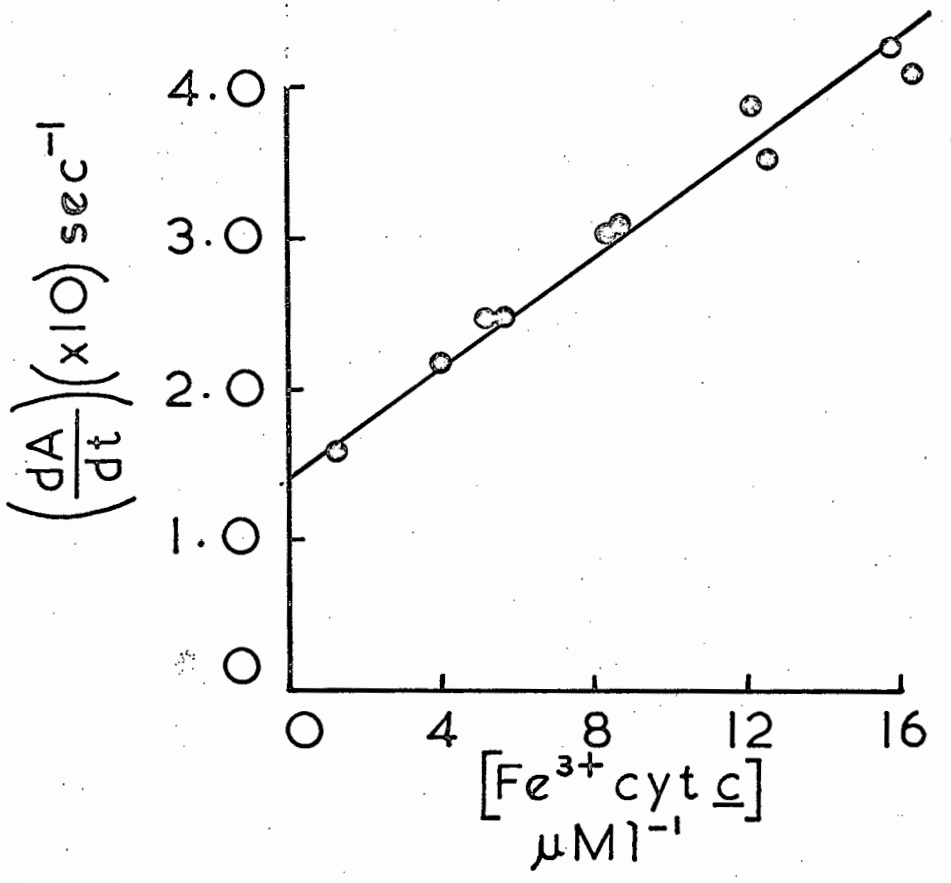

4.0
3.0
2.0
1.0
0
k(x10^7)
M^-1 sec^-1
0
10
20
30
40
[Fe^2+ cyt c](x10^6)

Fig. 16. Plot of log (Maximum Velocity) against log (Ferricytochrome *c*) for the reaction in phosphate buffer at pH 6.6.

Fig. 15. Plot of Maximum Velocity against concentration of added Ferricytochrome *c*, showing the influence of added product on the reaction in phosphate buffer at pH 6.6.


-2.3
-2.4
-2.5
-2.6
-2.7
log (dA/dt)
-5.5
-5.0
-4.5
log [Fe3+ cyt c]


4.0
3.0
2.0
1.0
0
(dA/dt) (x10) sec-1
0
4
8
12
16
[Fe3+ cyt c]
μM l-1

as indicating a half order dependence of the rate on ferricytochrome c. However, the effect observed when ferricytochrome c is added to a reaction is superimposed on the ordinary pseudo zero order reaction taking place, and this undoubtedly influences the slope obtained from fig. 16 , and consequently the observed order of the reaction with respect to ferricytochrome c is of little value.

It has already been shown that ferricytochrome c is modified by hydrogen peroxide. The possibility that the modified ferricytochrome c might cause the product activation of the reaction was investigated. 23μM ferrocytochrome c and 2.92×10^{-4}M hydrogen peroxide were used in reactions to which either 6.5μM of ferricytochrome c or 6.0μM of modified ferricytochrome c were added. In each case the maximum velocity was calculated. It was found that for the ferricytochrome c the velocity was $9.15 \times 10^{-7} Ml.^{-1} sec.^{-1}$, while for the modified ferricytochrome c the velocity was $2.44 \times 10^{-7} Ml.^{-1} sec.^{-1}$ compared with a rate of $1.85 \times 10^{-7} Ml.^{-1} sec.^{-1}$ in the absence of added product. These results show that it is the ferricytochrome c and not any modified form which is responsible for the product activation of the reaction.

The results of these experiments at pH 6.6 indicate that the rate constant is dependent on ferrocytochrome c

and hydrogen peroxide and that product activation occurs due to the formation of ferricytochrome c during the reaction. The reactions where the ferrocytochrome c concentration was varied gave a first order rate constant of $1.22 \times 10^{-2} sec.^{-1}$ (see fig. 14). This rate constant must be divided by the hydrogen peroxide concentration of $5.85 \times 10^{-4} M$ which had been regarded as a constant in these reactions, and is therefore present as part of the experimental rate constant. This gives a second order rate constant of 21 $l.M^{-1}sec.^{-1}$. Where the hydrogen peroxide concentration was varied, the slope of the plot of the experimental rate constant k against $[H_2O_2]$ (see fig. 12) gave a second order rate constant of 18 $l.M^{-1}sec.^{-1}$. The rate constant for the reaction between ferrocytochrome c and hydrogen peroxide, measured at pH 6.6, was therefore found to be of the order of 20 $l.M^{-1}sec.^{-1}$.

E. Attempt to Detect Free Radical Intermediates in the Reaction between Ferrocytochrome c and Hydrogen Peroxide.

Ferrocytochrome c is a single electron donor, and as the reaction between ferrocytochrome c and hydrogen peroxide requires two electrons to be transferred, it is likely that the reaction proceeds by two consecutive single electron transfer steps. This raises the probability of the formation of free radical intermediates,

due to the addition of only one electron to hydrogen peroxide. A similar intermediate has been proposed for the reaction between ferrous ions and hydrogen peroxide in aqueous solution by Barb[40] etal who assume the first step of their reaction scheme to be

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + HO^{\bullet}$$

The detection of such free radical intermediates during a reaction was investigated here. The usual methods employed by organic chemists to detect free radicals involve the use of compounds which are highly conjugated and can gain or lose electrons, forming free radicals, which are stabilized by the conjugation. This conjugation usually gives rise to the specific coloration of the compound which indicates the presence of free radicals. Unfortunately the majority of these compounds are non-polar and therefore are only soluble in non-polar solvents. Proteins are polar molecules and are therefore insoluble in non-polar solvents, thus making it difficult to use radical traps for reactions involving proteins. In addition there are a number of undesirable effects which prevent the use of radical traps in polar solvents, such as water.

Fridovich and Handler[41] have developed a method for determining qualitatively the presence of free radicals during enzymic oxidation. The method is based

on the ability of free radicals to initiate a chain reaction between sulphite ions and dissolved oxygen and does occur in aqueous solution. The oxygen uptake is measured to detect the presence of free radicals. This is usually achieved by the use of a sensitive oxygen electrode. As such an electrode was not available for this particular study, a Warburg manometer was used, the oxygen uptake being detected by change in the heights of the manometric fluid. 50μM Ferrocytochrome c, 0.005M sodium sulphite and 4ml. of phosphate buffer at pH 6.6 were placed in a Warburg flask and 5.85×10^{-4}M hydrogen peroxide was placed in the side arm. The Warburg manometer was flushed with oxygen for 5 minutes, equilibrated at 25°C in a thermostatted water bath and the initial readings taken. The reactions were then initiated by mixing the reactants and oxygen uptake was measured after 15 minutes. The results are recorded in table 2.

The results of Table 2 show that although a difference in manometer readings, corresponding to oxygen uptake was not detected in every case, in most of the reactions a small difference was observed. This suggests the presence of radical intermediates in the reaction.

Table 2. Manometric Measurement of Oxygen Uptake for Detection of Free Radicals by Method of Fridovitch.

Reaction	Initial Manometer Reading	Manometer Reading after 15 Minutes	Difference
Hydrogen peroxide blank	0.0cm.	0.0cm.	0.0cm
Ferrocytochrome c blank	0.1cm.	0.1cm.	0.0cm.
Fe^{2+}cyt c + H_2O_2	0.1cm.	0.6cm.	0.5cm.
Fe^{2+}cyt c + H_2O_2 -	0.1cm.	0.7cm.	0.8cm.
Fe^{2+}cyt c + H_2O_2	0.0cm.	0.0cm.	0.0cm.
Fe^{2+}cyt c + H_2O_2	0.0cm.	0.6cm.	0.6cm.

Following the first step of the reaction between ferrocytochrome c and hydrogen peroxide, the second step must be a reaction involving further ferrocytochrome c and the free radical produced from the first step. The free radical must be unstable due to the imbalance of electrons and its reaction with ferrocytochrome c is likely to be more rapid than that between ferrocytochrome c and hydrogen peroxide. The fact that the kinetic studies at both pH 8.1 and pH 6.6 show a first order dependence for ferrocytochrome c is evidence that the second step of the reaction is fast and therefore kinetically inaccessible.

An attempt was made to generate the free radical intermediates of the hydrogen peroxide, by ultraviolet

irradiation, to see if any increase in the rate of reaction could be observed, Reactions were run using 23μM ferrocytochrome c and 2.92×10^{-4}M hydrogen peroxide in phosphate buffer at pH 6.6, and irradiated by an ultraviolet lamp for a short period immediately after the reaction was initiated. The results are shown in Table 3 which shows that the ultravoilet radiation does increase the rate of reaction. This can be attributed to the dissociation of hydrogen peroxide into free radicals which react more rapidly with the ferrocytochrome c than the hydrogen peroxide itself.

Table 3. The Effect of Ultraviolet Radiation on the Reaction between Ferrocytochrome c and Hydrogen Peroxide at pH 6.6

Amount of Irradiation after Iniation of Reaction.	Total Time for Reaction	Maximum Velocity of Reaction
1. None	$2\frac{3}{4}$ minutes	1.60×10^{-7}M L^{-1}sec.$^{-1}$
2. 1 minute (high wavelength u.v. irradiation)	$2\frac{3}{4}$ minutes	1.62×10^{-7}M L^{-1}sec.$^{-1}$
3. $\frac{3}{4}$ minute (low wavelength u.v. irradiation)	2 minutes	2.42×10^{-7}M L^{-1}sec.$^{-1}$
4. $\frac{3}{4}$ minute (low wavelength u.v. irradiation)	$2\frac{1}{4}$ minutes	2.27×10^{-7}M L^{-1}sec.$^{-1}$

F. The Effect of Copper on the Reaction between Ferrocytochrome c and Hydrogen Peroxide

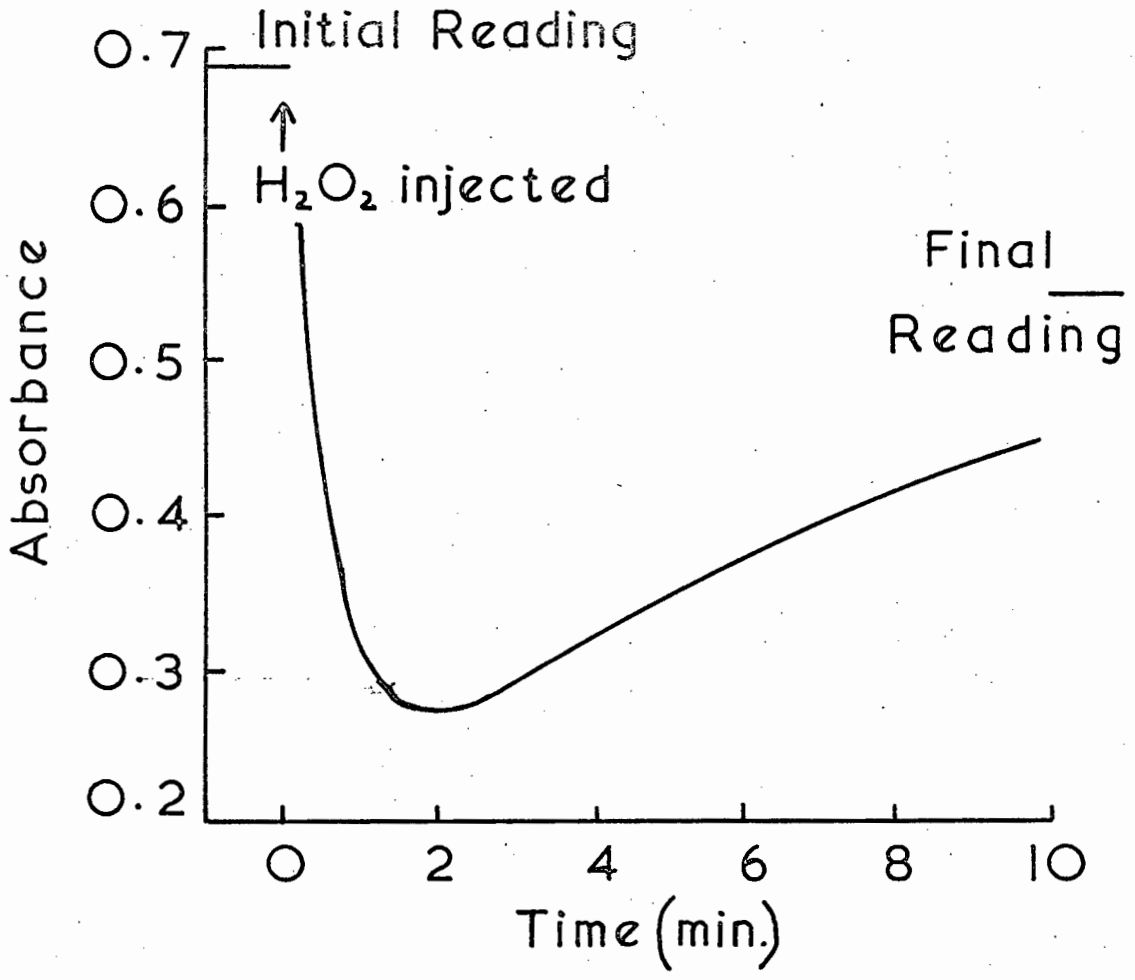
The effect of a copper histidine complex on the

reaction between ferrocytochrome c and hydrogen peroxide has been investigated to determine whether the copper catalyses the reaction.

The reactions were performed in Kolthoff phosphate buffer at pH 8.7, since under these conditions the reaction in the absence of copper was found to be slow. The presence of copper - histidine complex was found to increase the rate of oxidation of ferrocytochrome c considerably. When this oxidation was complete, a slow reduction of the oxidized cytochrome c was observed, which ceased after the cytochrome c was 60 - 70% reduced. (see fig. 17). This reduction of the cytochrome c was observed by scanning the absorption spectrum over the wavelength range 560mμ to 510mμ. These scans showed the formation of α and β peaks, identical with those of native ferrocytochrome c, eliminating the possibility of the reduced protein being some modified form of cytochrome c. When either only hydrogen peroxide or only copper - histidine complex was added to ferricytochrome c at pH 8.7 no reduction occurred, but when both were added to ferricytochrome c reduction occurred. This indicates that the reduction of ferricytochrome c at pH 8.7 is dependent on both hydrogen peroxide and copper - histidine complex.

Kinetic studies of the oxidation reaction were

Fig. 17. Typical reaction curve of Absorbance at 550mμ against time, for the reaction catalysed by copper ions in Kolthoff phosphate buffer at pH 8.7.


Initial Reading
H_2O_2 injected
Final Reading
Absorbance
0.7
0.6
0.5
0.4
0.3
0.2
0
2
4
6
8
10
Time (min.)

out in which the initial concentrations of each reactant were varied independently. The effect of ferrocytochrome c was determined, using 3.3×10^{-4}M copper - histidine complex and 2.92×10^{-4}M hydrogen peroxide and varying the ferrocytochrome c concentration between 11μM and 33μM. The results are shown in the plot of log (Initial Velocity) against log [Fe^{2+}cyt c] (see fig. 18) which indicates a first order dependence on ferrocytochrome c. The plot of the experimental rate constant k against [Fe^{2+}cyt c] (see fig. 19) has a negative slope which could indicate product inhibition, but this was disregarded because of insufficient evidence.

The evidence of both hydrogen peroxide and copper - histidine complex were determined, using the method previously adopted for determining the influence of hydrogen peroxide at pH 8.1 and pH 6.6. For the determination of the influence of hydrogen peroxide, 23μM ferrocytochrome c and 3.3×10^{-4}M copper - histidine complex were used and the hydrogen peroxide concentration was varied between 1.17×10^{-4}M and 5.85×10^{-4}M. The results are shown in fig. 20 where the plot of log (Initial Velocity) against log [H_2O_2] indicates a first order dependence on hydrogen peroxide. A plot of the experimental rate constant k against [H_2O_2] (see fig. 21), gave a straight line passing through the origin, indicating

Fig. 18. Plot of log (Initial Velocity) against log (Ferrocytochrome *c* concentration) for the reaction catalysed by copper ions in Kolthoff phosphate buffer at pH 8.7.

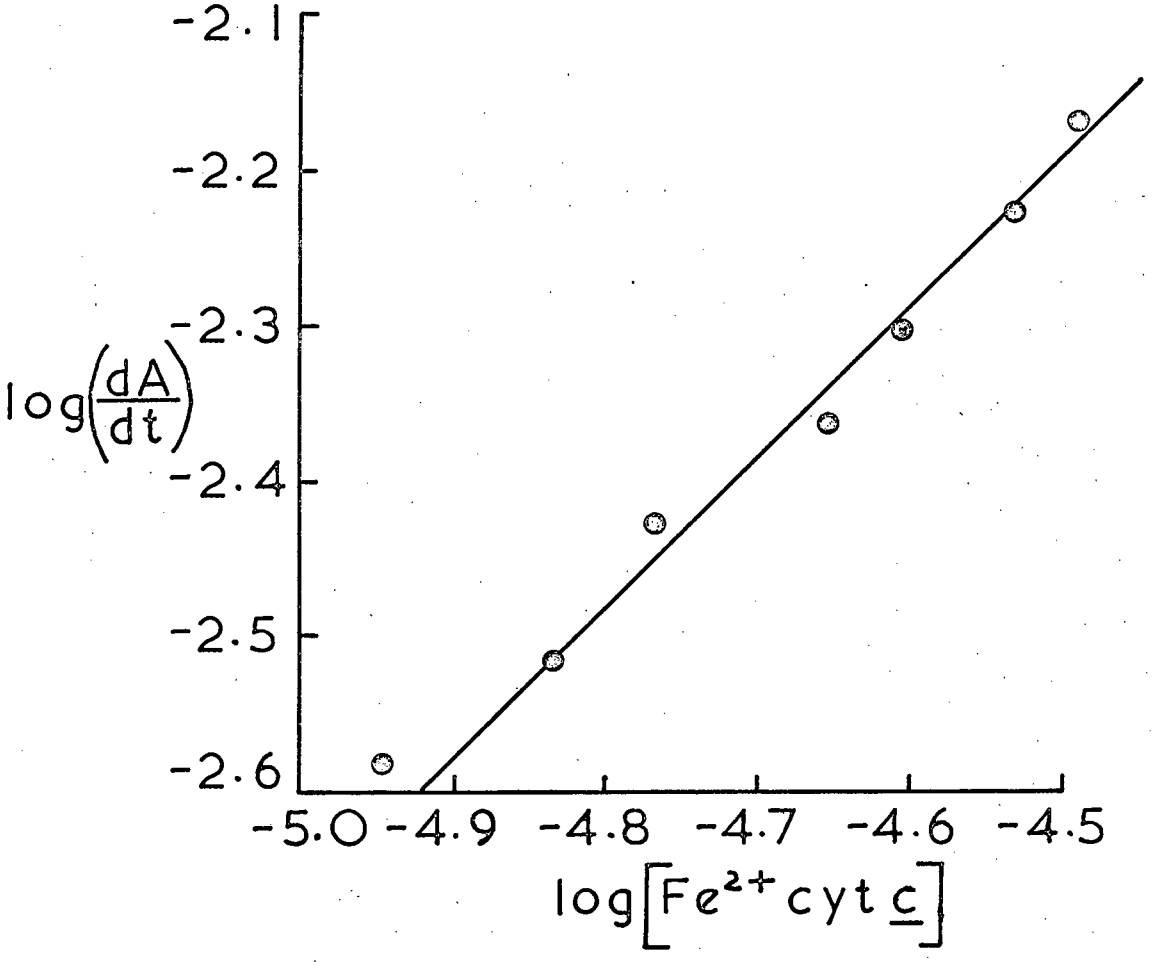
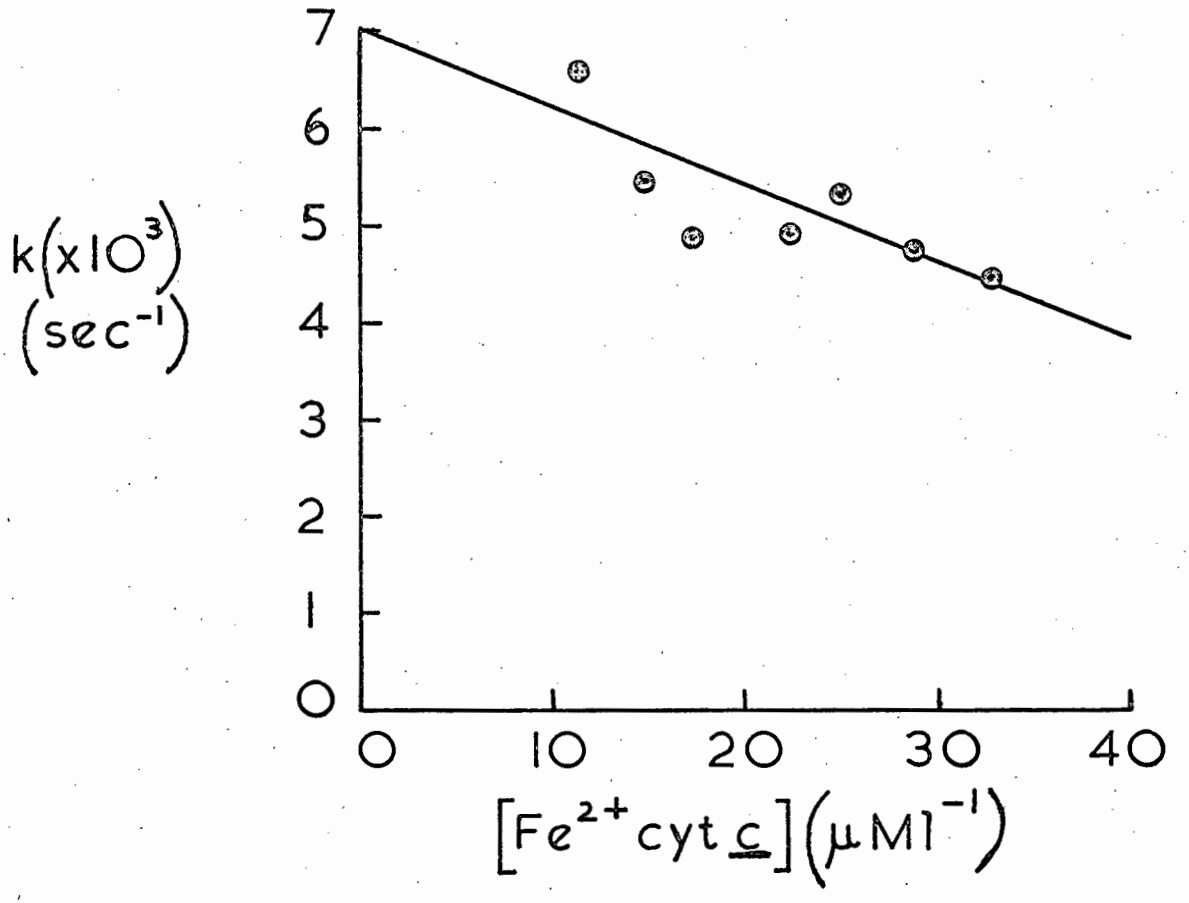

-2.1
-2.2
-2.3
-2.4
-2.5
-2.6
log(dA/dt)
-5.0
-4.9
-4.8
-4.7
-4.6
-4.5
log[Fe2+ cyt c]

Fig. 19. Plot of the Rate Constant against Ferrocytochrome c concentration for the reaction catalysed by copper ions in Kolthoff phosphate buffer at pH 8.7.


k(x10³) (sec⁻¹)
7
6
5
4
3
2
1
0
0
10
20
30
40
[Fe²⁺ cyt c] (μM)⁻¹

of activation might be determined.

The reaction at pH 8.1 was studied using 25μM ferrocytochrome *c* and 5.85×10^{-4}M hydrogen peroxide. The temperature was varied between 25°C and 40°C. The results were graphed in the Arrhenius plot of log k against $\frac{1}{T^{\circ}K}$ (see fig. 24). The slope of this graph was found to be - 3.5 (±0.5) which gave an activation energy E_a = 16 k.cals.mole^{-1} and an entropy of activation ΔS^{*}_{298} = -5e.u. The graph (fig. 24) shows a wide scatter of the points and the straight line was fitted by linear regression. An alternative interpretation is possible if separate lines are drawn for the points below 33°C and above 33°C, however this was only justified for one set of data. (fig. 24 set ▲).

The temperature study at pH 6.6 was performed using 20μM ferrocytochrome *c* and 5.85×10^{-4}M hydrogen peroxide. The temperature was varied between 25°C and 45°C. The results are recorded in the Arrhenius plot of log k against $\frac{1}{T^{\circ}K}$ (see fig. 25) which gave a straight line of slope - 2.5. From this the activation energy was calculated to be E_a = 11.5 k.cal.mole^{-1} and the entropy of activation was found to be ΔS^{*}_{298} = -15e.u.

The reaction at pH 8.7 was carried out in Kolthoff phosphate buffer, using 23μM ferrocytochrome *c*, 2.92×10^{-4}M hydrogen peroxide and 3.3×10^{-4}M copper

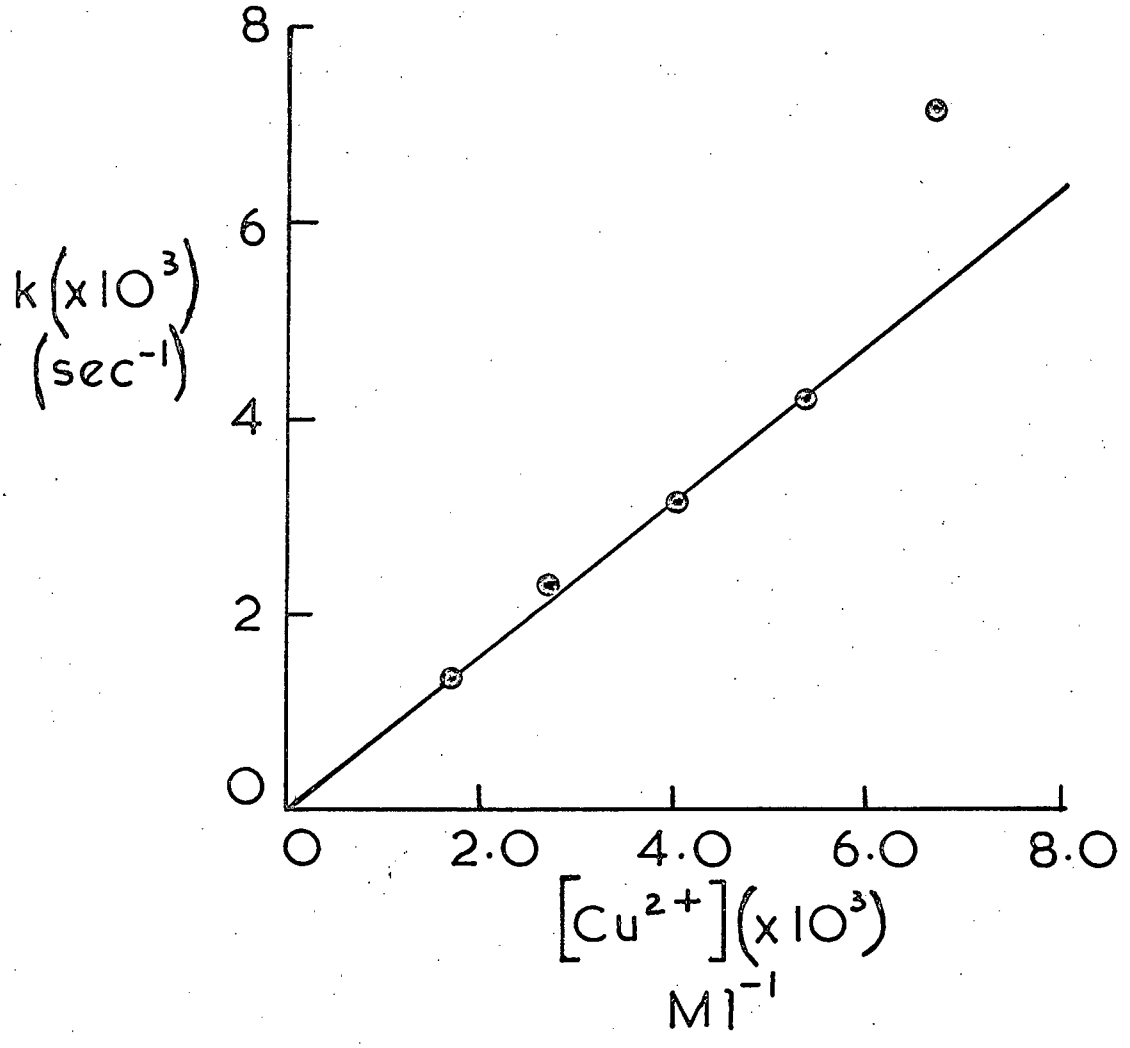

8
6
4
2
0
k (x10³) (sec⁻¹)
0
2.0
4.0
6.0
8.0
[Cu²⁺] (x10³) Ml⁻¹

Fig. 23. Plot of the Rate Constant against Copper ion concentration for the reaction catalysed by copper ions in Kolthoff phosphate buffer at pH 8.7.

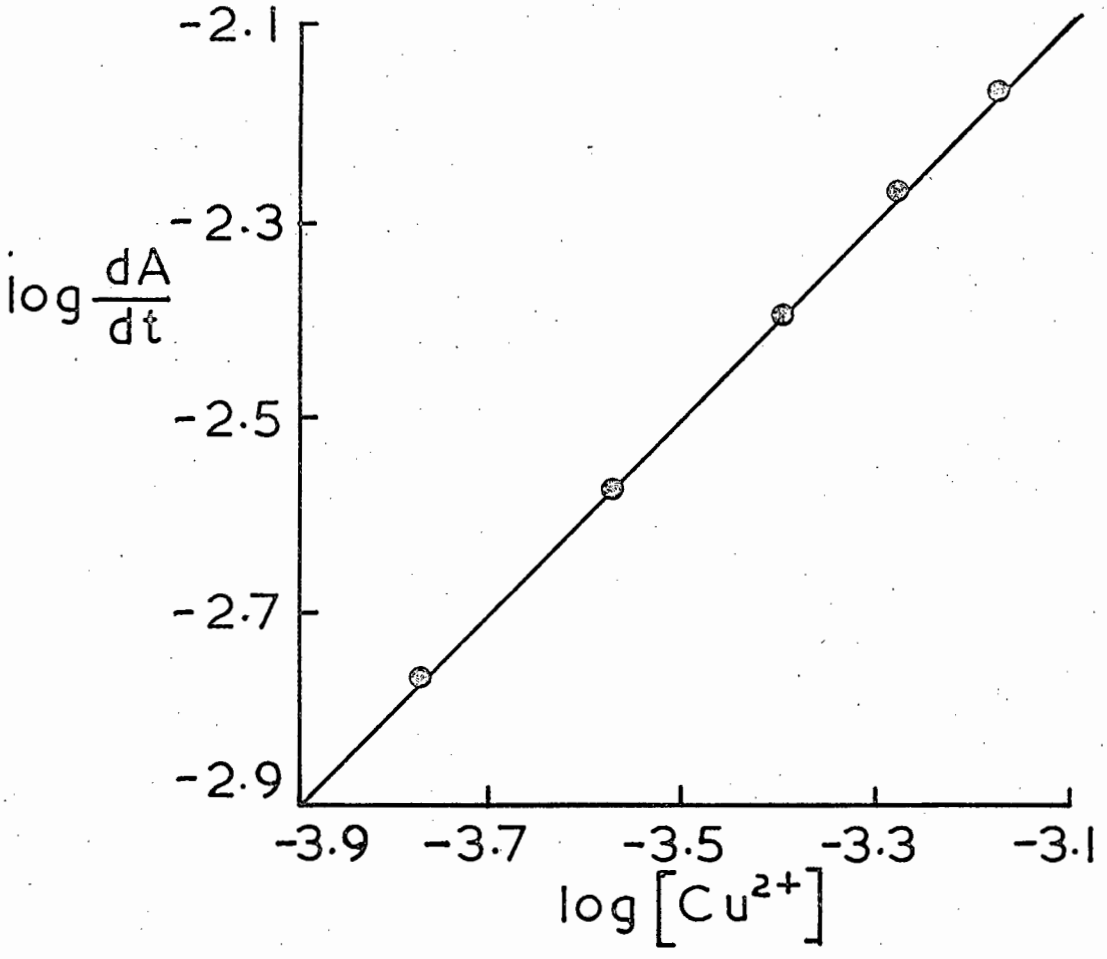

-2.1
-2.3
-2.5
-2.7
-2.9
log dA/dt
-3.9
-3.7
-3.5
-3.3
-3.1
log [Cu2+]

Fig. 22. Plot of log (Initial Velocity) against log (Copper ion concentration) for the reaction catalysed by copper ions in Kolthoff phosphate buffer at pH 8.7.

a simple relationship between the hydrogen peroxide concentration and the rate of the reaction.

The effect of copper - histidine complex on the reaction was determined using 25μM ferrocytochrome c and 1.4×10^{-4}M hydrogen peroxide, the copper - histidine concentration being varied between 1.6×10^{-4}M and 6.7×10^{-4}M. The results (see fig. 22) show a first order dependence of rate on copper - histidine complex. A plot of the experimental rate constant k against [Cu^{2+}- histidine], (see fig. 23), gives a straight line passing through the origin, indicating a simple dependence of rate on copper - histidine complex.

These kinetic results indicate that the reaction, catalysed by the copper complex, has a simple first order dependence on each of ferrocytochrome c, hydrogen peroxide and copper - histidine complex. The third order rate constant for the reaction was determined from these experimental results and found to be of the order of $5 \times 10^{4} l^{2} M^{-2} sec^{-1}$.

G. The Effect of Temperature on the Reaction between Ferrocytochrome c and Hydrogen Peroxide.

The effect of temperature on the reaction between ferrocytochrome c and hydrogen peroxide at pH 8.1, pH 6.6 and on the reaction catalysed by copper at pH 8.7, was studied so that the activation energies and entropies

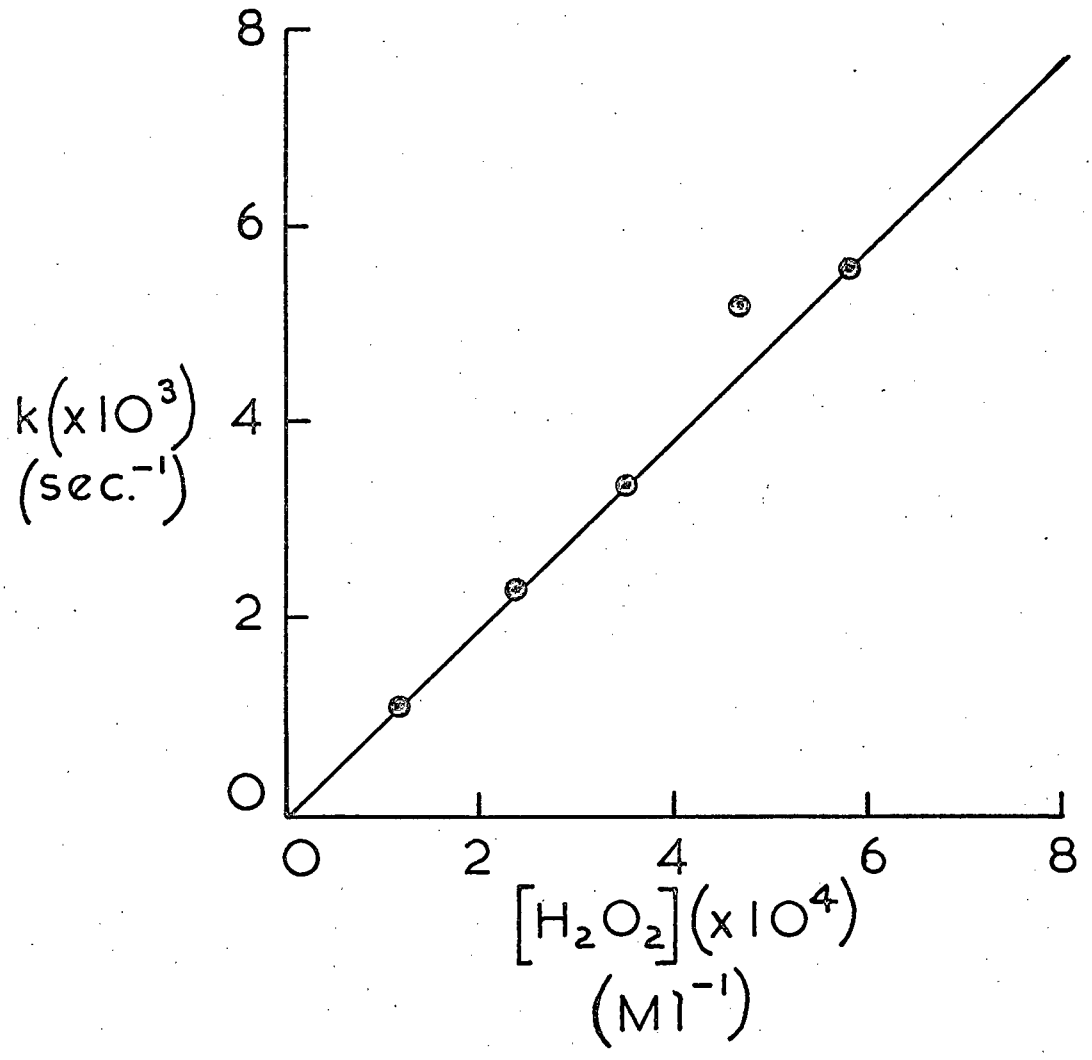

8
6
4
2
0
k(x10³)
(sec.⁻¹)
0
2
4
6
8
[H₂O₂](x10⁴)
(M1⁻¹)

Fig. 21. Plot of the Rate Constant against Hydrogen Peroxide concentration for the reaction catalysed by copper ions in Kolthoff phosphate buffer at pH 8.7.

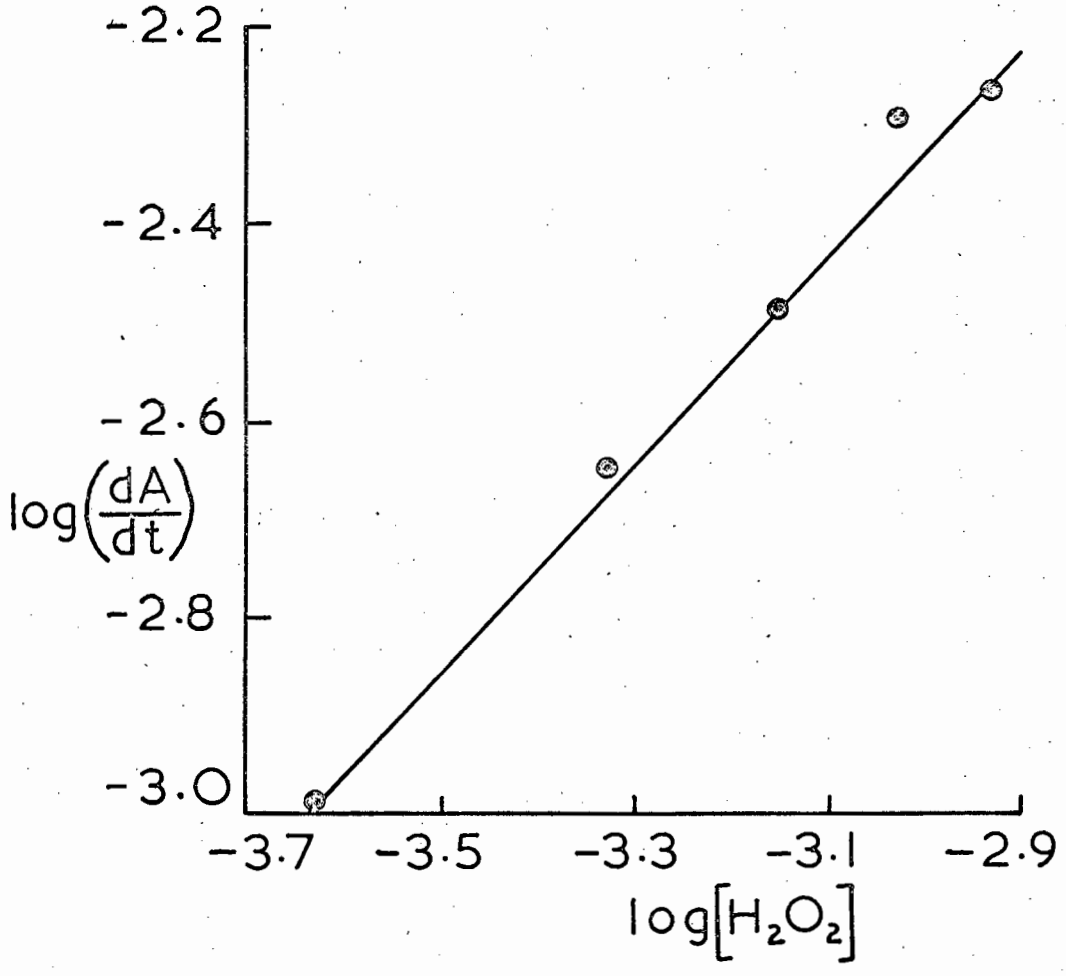

-2.2
-2.4
-2.6
-2.8
-3.0
log(dA/dt)
-3.7
-3.5
-3.3
-3.1
-2.9
log[H2O2]

Fig 20. Plot of log (Initial Velocity) against log (Hydrogen Peroxide concentration) for the reaction catalysed by copper ions in Kolthoff phosphate buffer at pH 8.7.

and pH 7.9 the order of the reaction changes. Davison and Hamilton[23], using Theorell-Stenhagen buffer[42] in their study of pH effects on the reaction, observed that the order of reaction changed above pH 7.8. However, while they found that the rate of reaction decreased as the pH increases above pH 7.8, they noted an increase in rate with increasing pH below pH 7.8 and an optimum pH at which the maximum rate was greatest, at pH 8. This result is in conflict with those obtained in this study, the difference possibly being due to the different buffer systems used in the two studies.

The effect of pH on the reaction rate might be due to neutralization of the hydroxyl ions being formed during the reaction, by the presence of the hydrogen ions. However the ionic strength study indicates that little charge is created before the rate determining step, therefore it is unlikely that the hydroxyl ion is formed before the rate determining step, as this involves the creation of charge. Hydrogen ion concentration is known to influence reactions of cytochrome c since the different ionized forms of the molecules differ in their reactivity towards many reagents. Evidently hydrogen ions can be excluded as a reactant in the reaction, which is in contrast with the autoxidation studies of Boeri and Tosi[19] who found a dependence of rate on the hydrogen

IV. DISCUSSION

A. Physical Factors

(1) Effect of Ionic Strength and Hydrogen Ion Concentration.

The study of the influence of ionic strength at pH 6.6 shows that the rate of reaction increased as the ionic strength of the solution was increased, over the range of ionic strengths studied. This increase was small, the maximum velocity of the reaction increasing from 2.38×10^{-7} M $l.^{-1}$ $sec.^{-1}$ to 2.84×10^{-7} M $l.^{-1}$ $sec.^{-1}$ for a corresponding change in ionic strength from $\mu = 0.013$ to $\mu = 0.170$. The net charge created during the formation of the transition state is given by the slope of the plot of log k against $\sqrt{\mu}$ (see fig. 4) and this was found to be + 0.5. This is a small value for the total charge formed. The increase in velocity does not suggest a direct dependence of rate on the ionic strength, such as that found by Boeri and Tosi[19] for the autoxidation of ferrocytochrome c. The low value of + 0.5 for the total charge created in the formation of the transition state, suggests that the rate determining step of the reaction occurs early in the reaction, before the hydrogen peroxide has been able to dissociate into ions and radicals.

It has been observed that as the pH increases, the reaction rate decreases and that between pH 7.6 and

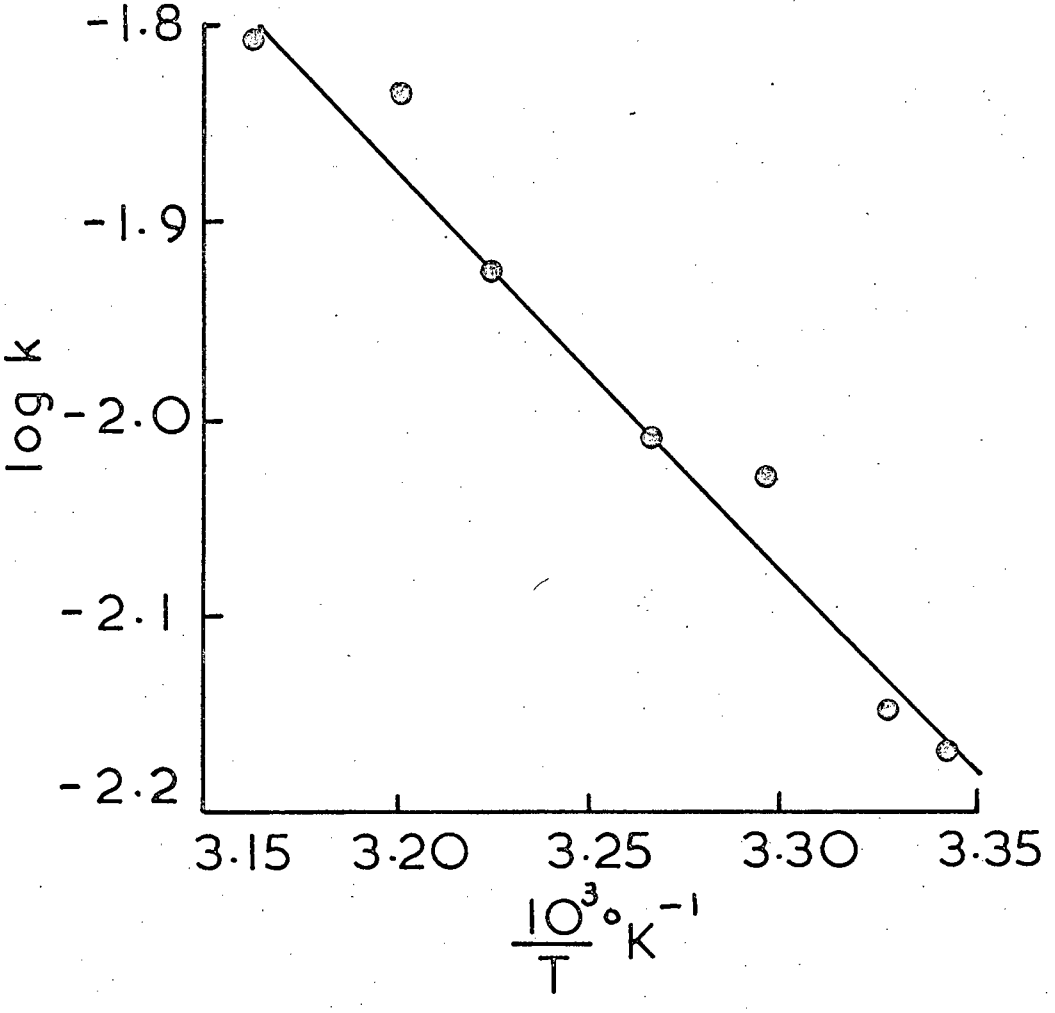

-1.8
-1.9
-2.0
-2.1
-2.2
log k
3.15
3.20
3.25
3.30
3.35
10³°K⁻¹
T

Fig. 26. Arrhenius plot of log k against $\frac{10^3}{T}$ $^oK^{-1}$ for the reaction catalysed by copper ions in Kolthoff phosphate buffer at pH 8.7.

histidine complex. The temperature was varied between 25°C and 43°C. The Arrhenius plot of log k against $\frac{1}{T^{o}K}$ (see fig. 26) shows the results obtained. This plot gave a straight line of slope - 2 from which an activation engergy E_a = 9 k.cal.mole.$^{-1}$ and an entropy of activation of ΔS^{*}_{298} = - 8e.u. was calculated.

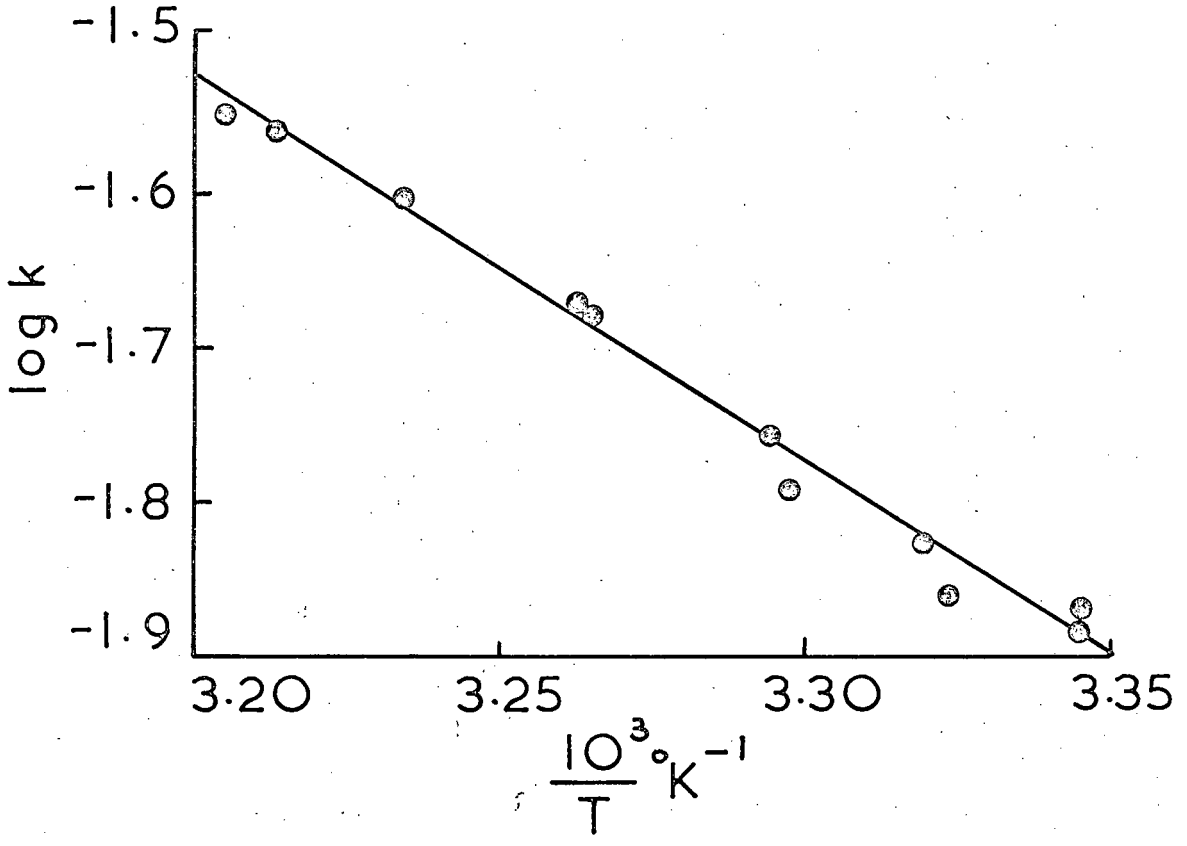

-1.5
-1.6
-1.7
-1.8
-1.9
log k
3.20
3.25
3.30
3.35
10³/T °K⁻¹

Fig. 25. Arrhenius plot of log k against $\frac{10^3}{T}$ $^oK^{-1}$ for the reaction in phosphate buffer at pH 6.6.

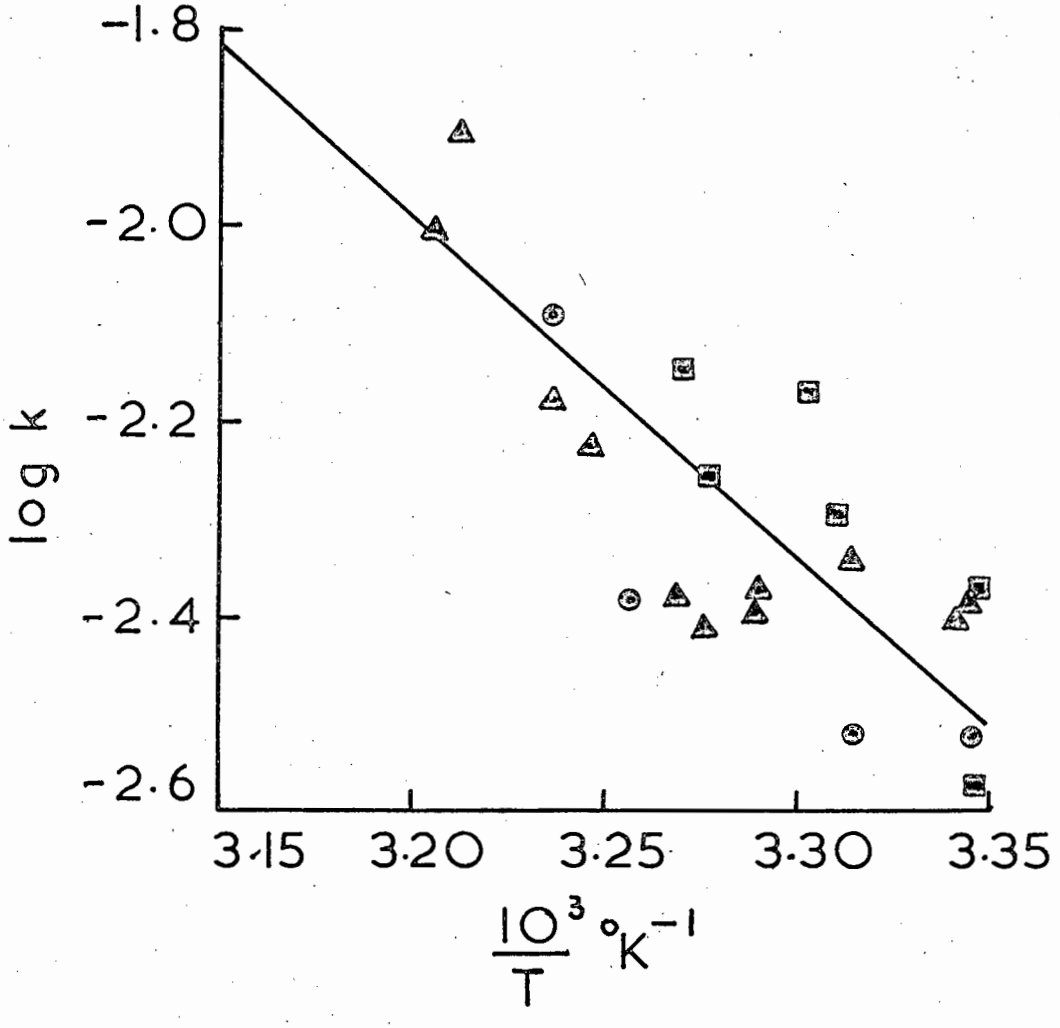

-1.8
-2.0
-2.2
-2.4
-2.6
log k
3.15
3.20
3.25
3.30
3.35
10³ °K⁻¹
T

Fig. 24. Arrhenius plot of log k against $\frac{10^3}{T}$ o_K-1 for the reaction in phosphate buffer at pH 8.1.

ion concentration, as well as the ionic strength.

The changes in ferrocytochrome c which influence its reactivity have been attributed by some workers, including Greenwood[14], chiefly to changes in pH, while other workers, Skov and Williams[15] have suggested that these changes are determined rather by the ionic strength of the solution. It was therefore of interest to know whether the changes in reactivity observed in this study, between pH 7.6 and pH 7.9, were due to either pH or ionic strength or both. The ionic strength has been found to have only a small influence on the rate at pH 6.6, and when increased from $\mu = 0.013$ to $\mu = 0.17$, no measurable change in the order of the reaction was observed. When the pH value was changed from pH 6.6 to pH 8.1, the accompanying change in ionic strength was $\mu = 0.03$ to $\mu = 0.07$, which is a much smaller change than that for which no change in the reactivity was observed at pH 6.6. Further, the reaction was carried out in Kolthoff phosphate buffer, over the same pH range and the changes in reactivity occurred at the same pH values. This tends to favour the hydrogen ion concentration as the main contributing influence on the change of reactivity, although the influence of ionic strength cannot be dismissed and might have a secondary effect.

The influence of the hydrogen ion concentration in the range pH 7.6 - 7.9 is of interest since a change in the order of reaction is noted in this range. This is probably caused by the ionization of an amino acid residue situated close to the reactive site of the cytochrome c. None of the usual ionizations of free amino acids (such as those of carboxylic groups, amino groups or ring nitrogens) have pK values in the region of pH 7 to 8, the nearest being the imidazole of histidine, pK = 6 and the - SH of cysteine, pK = 8.3. However in proteins, the pK values of the amino acids are altered by their environment and accessibility to solvent. The calculated pK values for imidazole, and for the terminal alpha NH_2 of proteins are 7.0 and 7.8 respectively. In ribonuclease these values have been experimentally determined by Tanford[43] to be 6.5 and 7.8 respectively. Rodkey and Ball[17] have studied the redox potentials of cytochrome c and observed a change of slope for the plot of E_o' against pH occurring at pH 7.8. This observed ionization is consistent with either a hydrogen ion dissociating from, or a hydroxyl ion being added to cytochrome c, the pK value for either such process being 7.8.

This ionization could be responsible for the change in reactivity which occurs. It is possible that the

cytochrome c has a site which is blocked and therefore inactive, when a hydrogen ion is associated to it, but which becomes active on dissociation of the hydrogen ion. Thus the cytochrome c might be associated to the hydrogen above pH 7.8, preventing the ferricytochrome c from acting as a catalyst, while below pH 7.8 the hydrogen ion dissociates from the cytochrome c and the ferricytochrome c is in an active form, having a site available for catalysis of the reaction.

The effect of hydrogen ions on the reaction rate did not follow a simple order of reaction, and the results were therefore analysed on the assumption that a pH dependent equilibrium existed between a reactive (protonated) form, represented as $Fe^{2+}cyt\underline{c}\ H^+$ and an unreactive, dissociated form, represented as $Fe^{2+}cyt\underline{c}$. consequently

$$K_a = \frac{[H^+]\,[Fe^{2+}cyt\underline{c}]}{[Fe^{2+}cyt\underline{c}\ H^+]}$$

The total concentration of ferrocytochrome c equals the sum of the concentrations of the associated and dissociated forms, i.e.

$$[Fe^{2+}cyt\underline{c}_{tot}] = [Fe^{2+}cyt\underline{c}] + [Fe^{2+}cyt\underline{c}\ H^+]$$

These two equations are combined with the rate equation

$$v = k\,[Fe^{2+}cyt\underline{c}\ H^+]$$

to give the equation

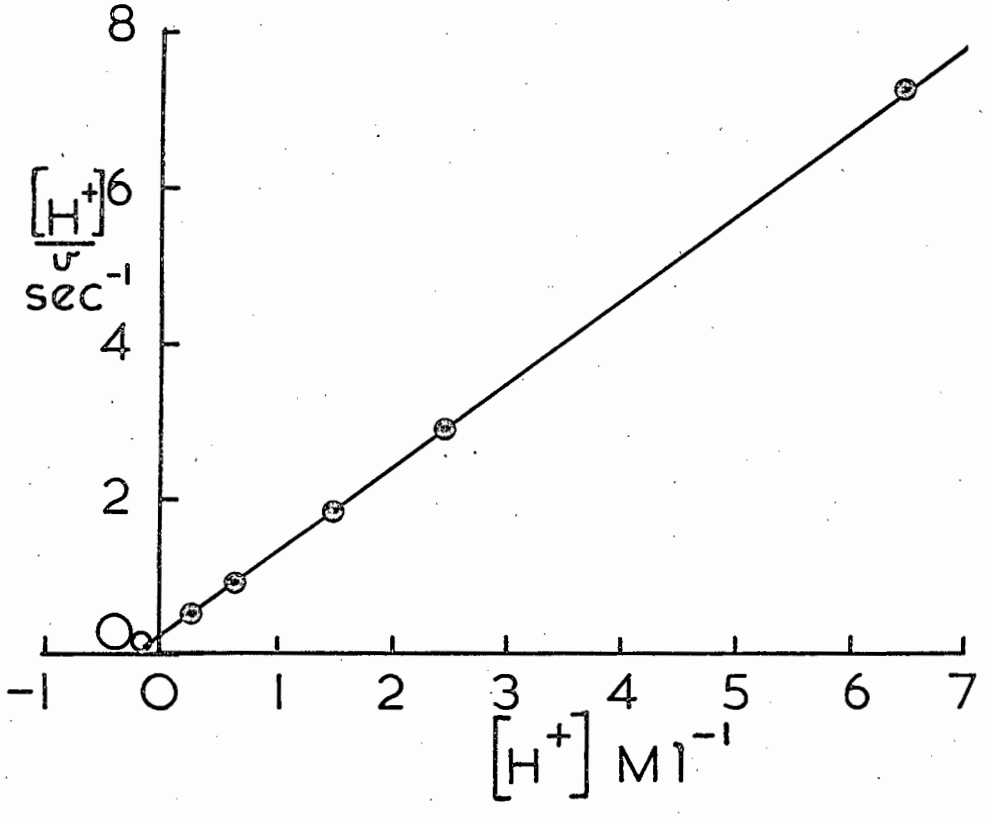
$$\frac{[H^+]}{v} = \frac{K_a}{k[Fe^{2+}cyt\underline{c}_{tot}]} + \frac{[H^+]}{k[Fe^{2+}cyt\underline{c}_{tot}]}$$

If this relationship is valid then a plot $\frac{[H^+]}{v}$ against $[H^+]$ should give a straight line whose intercept with the $[H^+]$ axis is equal to $- K_a$. The results from fig. did indeed form a straight line when plotted in this way (see fig. 27) and the intercept was found by a linear regression of the results, to be $- 1.9(\pm 0.2) \times 10^{-8}$ which is equivalent to a pK value of about 7.7. The linearity of the relationship supports the hypothesis that the cause of the change in the reactivity between pH 7.6 and 7.9 is a dissociation of a hydrogen ion from a site in the cytochrome c molecule having a pK equal to 7.8. The agreement, within experimental error, with the value of Rodkey and Ball[17] further suggests that protonation of the same reactive site is also important in determining the redox potential of the molecule.

(2) The Influence of Temperature on the Reaction between Ferrocytochrome c and Hydrogen Peroxide

Increasing the temperature at which the reactions were carried out was found to increase the rate of reaction for the studies at pH 8.1, pH 6.6 and for the copper catalysed reaction at pH 8.7. From Arrhenius

Fig. 27. Plot of $\frac{[H^+]}{v}$ against $[H^+]$ to determine the effect of hydrogen ion concentration and to obtain the value of K_a.


8
6
4
2
$\frac{[H^+]}{v}$ sec^{-1}
−1
0
1
2
3
4
5
6
7
$[H^+]$ M l^{-1}

plots for all these reactions, activation energies, enthalpies of activation and entropies of activation were calculated. These results are summarised in Table 4.

Table 4. Summary of Values for Activation Energies and Enthalpies and Entropies of Activation

pH	Reaction	E_a (k.cal.mole.$^{-1}$)	ΔH^*_{298} (k.cal.mole.$^{-1}$)	ΔS^*_{298} (e.u.)
8.1	Fe^{2+}cyt<u>c</u> + H_2O_2	16	15	- 5
6.6	Fe^{2+}cyt<u>c</u> + H_2O_2	11.5	11	-15
8.7	Fe^{2+}cyt<u>c</u> + H_2O_2 + Cu^{2+}	9	8	- 8

The enthalpy of activation represents the amount of energy required to form the transition state from the reactants. In the reactions studied, it is improbable that the enthalpy of activation represents simple interactions, particularly as catalytic influences are present at both pH 6.6 and pH 8.7. The value for the enthalpies of activation therefore represent the energy necessary to overcome the highest of several such barriers in the system. This prevents the assignment of the enthalpy of activation to any particular event occurring during the reaction.

Similarly, the entropy of activation, for systems as complex as those studied here, is the sum of

contributions from several occurrences during the formation of the transition state. This precludes any meaningful analysis of the calculated entropies of activation.

It is, however, interesting to compare the values obtained for the activation energy under the various conditions used in this study, and also to compare these values with others obtained for similar systems by other workers.

The reaction at pH 8.1 has a higher activation energy than the reaction at pH 6.6 or the reaction at pH 8.7. Catalysts function by lowering free energies of activation. The reaction at pH 6.6 is thought to be autocatalytic and was found to have a lower activation energy than the reaction at pH 8.1. Similarly, the reaction at pH 8.7 catalysed by copper was found to have a lower activation energy. Presumably therefore the catalytic effects at pH 6.6 and in the presence of copper are due more to effects on the enthalpy term in the free energy of activation than to effects of entropies of activation, which do not appear in the Arrhenius activation energies calculated here. However, in contrast, Shashoua[24] has obtained a value of 3.5 k.cal.mole^{-1} for the activation energy of the cytochrome c - hydrogen peroxide reaction at pH 9. It

is conceivable that the activation energy decreases as the pH increases from pH 8 to pH 9 for the uncatalysed reaction, and that the decrease reported above for copper at pH 8.7 is not solely due to the copper.

The value of the activation energy for the reaction at pH 8.1, where no catalytic influences are thought to be present, can be compared with the activation energy of 10 k.cal.mole^{-1} obtained by Baxendale [27] etal for the ferrous ion - hydrogen peroxide reaction. This suggests that the protein - porphyrin portion of the cytochrome c molecule tends to increase the height of the energy barrier, presumably because the iron is found in the cytochrome c molecule in such a way that it is less accessible than the ferrous ions, to oxidation by the hydrogen peroxide. The physiological function of cytochrome c in energy production is dependent on this property, that ferrocytochrome c is not easily oxidized, except in the presence of the enzyme cytochrome oxidase. When a molecule of cytochrome c is oxidized in a cell by cytochrome oxidase, a molecule of ATP is generated which the cell can use to provide energy for its life processes.

B. Mechanisms for the Reaction between Ferrocytochrome c and Hydrogen Peroxide

The kinetic consequences of the reactions at pH 8.1, pH 6.6 and for the copper catalysed reaction were determined and an attempt was made to fit suitable reaction mechanisms to the observed results. This was limited to reasonably simple mechanisms since the results, with the exception of those at pH 6.6, did not require the use of more complex mechanisms.

In comparing maximum rates, a first order dependence on ferrocytochrome c was found. This indicates that only one molecule of ferrocytochrome c was involved in the rate determining step of the reaction. However the overall reaction involves a two electron transfer from ferrocytochrome c to the hydrogen peroxide and therefore two molecules of ferrocytochrome c must be involved in the overall reaction. The reaction between ferrous ions and hydrogen peroxide can be compared with the reaction studied here. As was noted in the introduction, this reaction involves the two reaction steps

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \qquad \ldots\ldots(1)$$

$$Fe^{2+} + {}^{\bullet}OH \longrightarrow Fe^{3+} + OH^- \qquad \ldots\ldots(2)$$

and was found to obey the rate equation

$$-\frac{d[Fe^{2+}]}{dt} = 2k[Fe^{2+}][H_2O_2] \quad \ldots.(3)$$

The rate equation (3) for the reaction suggests that the reaction step (2) is rapid and therefore kinetically inaccessible. This assumes that the intermediate hydroxyl radical formed as a product of (1) is a highly active species which will undergo rapid interaction with a ferrous ion. This assumption can similarly be used in the case of the reaction between ferrocytochrome c and hydrogen peroxide. One of the products of the initial reaction between the protein and hydrogen peroxide is likely to be a hydroxyl radical which will undergo a rapid reaction with ferrocytochrome c since it is a highly active intermediate. As this step is rapid it will be kinetically inaccessible.

The observed acceleration of the reaction by ultraviolet irradiation supports this proposal. The effect of ultraviolet radiation on aqueous solutions of hydrogen peroxide has been studied by P. Smith(44), who proposed that the first step of the resulting decomposition is the homolytic cleavage of hydrogen peroxide into two hydroxyl radicals.

$$H_2O_2 + h\nu \longrightarrow 2^{\bullet}OH$$

Thus the effect of ultraviolet radiation on the reaction between ferrocytochrome c and hydrogen peroxide is to increase the concentration of hydroxyl radicals in the

solution and thus to increase the overall rate of the reaction.

The reaction between ferrocytochrome c and the hydroxyl radical was thus regarded as a rapid interaction and was kinetically inaccessible. This accounts for the dependence of the rate on ferrocytochrome c being first - and not second - order.

(1) The Reaction at pH 8.1

The analysis of the kinetic studies made at pH 8.1 indicates that the rate of reaction has a simple first order dependence on both the reactants, ferrocytochrome c and hydrogen peroxide. Also the reaction does not appear to be subject to either product inhibition or product activation. The results obey the simple rate equation

$$-\frac{d[Fe^{2+}cyt\underline{c}]}{dt} = k\,[Fe^{2+}cyt\underline{c}]\,[H_2O_2]$$

A simple mechanism can therefore be fitted to the experimental results of the kinetic analysis. The mechanism proposed was

$$Fe^{2+}cyt\underline{c} + H_2O_2 \xrightarrow{k} Fe^{3+}cyt\underline{c} + {}^{\bullet}OH + OH^{-}$$

$$Fe^{2+}cyt\underline{c} + {}^{\bullet}OH \xrightarrow{fast} Fe^{3+}cyt\underline{c} + {}^{-}OH$$

which although appearing satisfactory is very likely an oversimplification of what actually occurs during

the reaction. This is suggested by the occasional deviation from a slope of zero observed for the plot of the experimental rate constant k against [Fe^{2+}cyt<u>c</u>] (see fig. 7).

(2) The Reaction at pH 6.6

The kinetic studies of the reaction between ferrocytochrome <u>c</u> and hydrogen peroxide at pH 6.6 indicate that a simple first order dependence holds for both ferrocytochrome <u>c</u> and hydrogen peroxide. The reaction is, however, more complicated as suggested by the difference between the true order of the reaction with respect to ferrocytochrome <u>c</u> and the order with respect to time.

Three possible explanations of why this difference should occur in the kinetics are inhibition by substrate, activation by intermediate, probably free radicals, or activation by products of the reaction. The substrate inhibition seems unlikely since increasing the substrate (ferrocytochrome <u>c</u>) concentration, increases the rate proportionally, which would not be expected if substrate inhibited the reaction. The hydroxyl radical has been proposed as a very active intermediate and therefore is unlikely to accumulate to a suitable concentration to allow it to activate the reaction. Product activation is proposed by Laidler[38] as a likely explanation for

such observed differences in the kinetics. When ferricytochrome c was added to the reaction, the rate increased proportionally to the amount added. (see fig.15) Thus product activation is responsible for the differences arising between the 'true order' and the order with respect to time observed at pH 6.6.

Thus all mechanisms proposed to explain the kinetics must account for product activation and must be able to produce a plot of [Fe^{2+}cyt c] against time similar to the experimental reaction curves (see fig. 2). Although several mechanisms were tried, none of those tested were found to fit the experimental results completely satisfactorily. The mechanisms which were tested and gave the best fits are discussed below.

(a) If the rapid formation of an intermediate between ferrocytochrome c and hydrogen peroxide takes place and this is followed by an interaction between the intermediate and ferricytochrome c, then the mechanism can be written as

$$Fe^{2+}\text{cyt}\,c + H_2O_2 \underset{}{\overset{\text{fast}}{\rightleftharpoons}} (Fe^{2+}\text{cyt}\,c \cdot H_2O_2)$$

$$(Fe^{2+}\text{cyt}\,c \cdot H_2O_2) + Fe^{3+}\text{cyt}\,c \xrightarrow{k_{+2}} 2Fe^{2+}\text{cyt}\,c + \text{products}$$

This mechanism gives the rate equation

$$-\frac{d[Fe^{2+}\text{cyt}\,c]}{dt} = k_{+2}\,[Fe^{2+}\text{cyt}\,c]\,[Fe^{3+}\text{cyt}\,c]\,[H_2O_2].$$

When the hydrogen peroxide is in excess, its concentration

can be treated as a constant and be included with the rate constant to give

$$-\frac{d[Fe^{2+}cyt\underline{c}]}{dt} = k'_{+2}\,[Fe^{2+}cyt\underline{c}]\,[Fe^{3+}cyt\underline{c}]$$

The total concentration of cytochrome c in the reaction system at any given time is constant and equal to the sum of the ferrocytochrome c and ferricytochrome c present, i.e.

$$[Fe^{2+}cyt\underline{c}_{tot}] = [Fe^{2+}cyt\underline{c}] + [Fe^{3+}cyt\underline{c}]$$

$$\therefore -\frac{d[Fe^{2+}cyt\underline{c}]}{dt} = k'_{+2}\,[Fe^{2+}cyt\underline{c}][Fe^{2+}cyt\underline{c}_{tot}] - k'_{+2}[Fe^{2+}cyt\underline{c}]^2$$

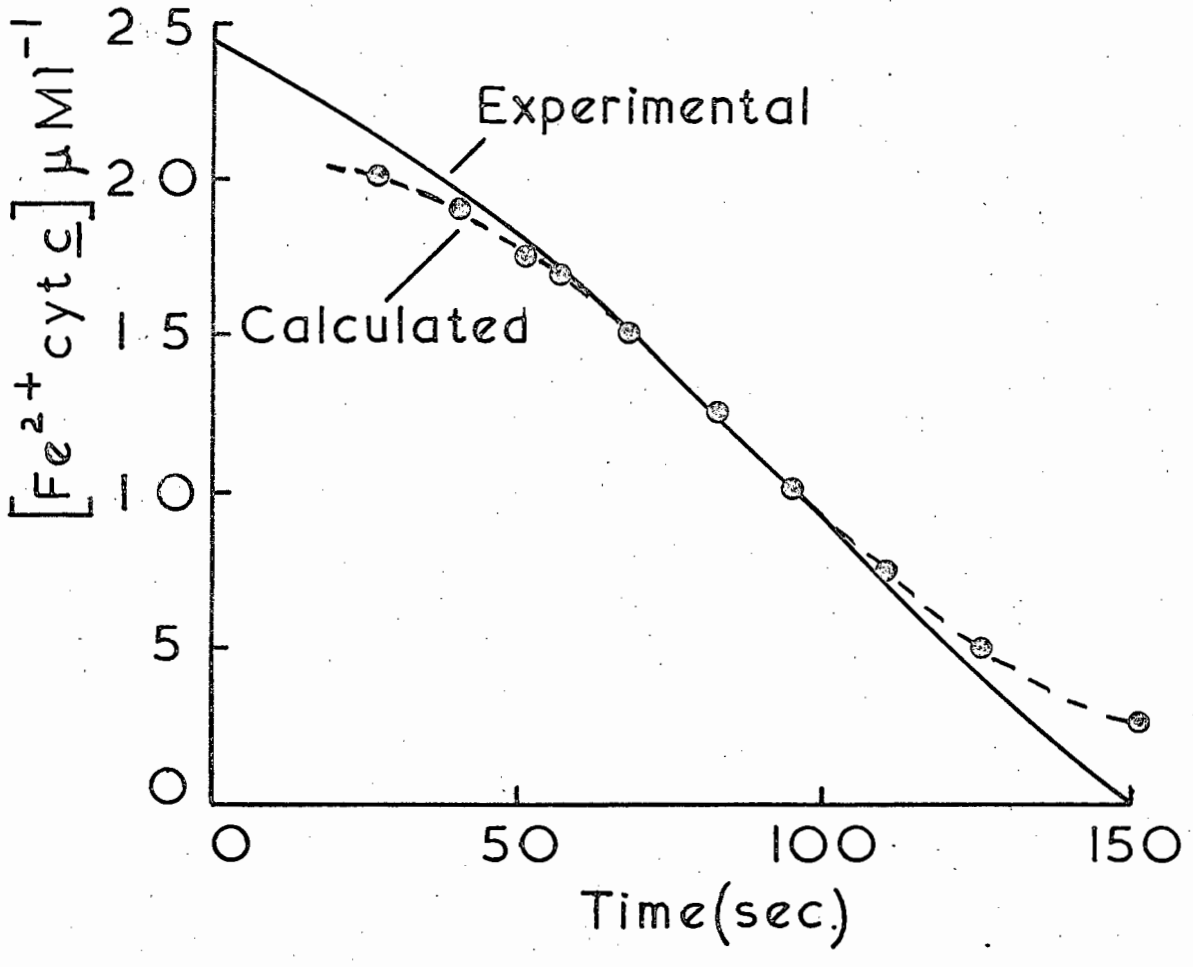
Integration of this rate equation gives the relationship between the ferrocytochrome c concentration and time as

$$t = \frac{2.303}{k'_{+2}[Fe^{2+}cyt\underline{c}_{tot}]} \log \frac{[Fe^{3+}cyt\underline{c}]}{[Fe^{2+}cyt\underline{c}]} + C$$

where C is a constant of integration.

Using experimental values for the concentration of ferrocytochrome c, corresponding time values were calculated and with a suitable value for the constant of integration were plotted and compared with the experimental reaction curves (see fig. 28). This mechanism produces a good fit for the intermediate region of the reaction, but the extremes show deviation from the

Fig. 28. Plot of Ferrocytochrome c concentration against time to compare results calculated from a proposed mechanism with those obtained by experiment.


[Fe2+ cyt c] μM)-1
25
20
15
10
5
0
Experimental
Calculated
0
50
100
150
Time(sec.)

experimental results. This mechanism was thus only consistent with the portion of the reaction in which steady state conditions might be expected to prevail.

(b) Other mechanisms were considered which produced similar results to the one above. A mechanism similar to that tried above, but including direct reaction between ferrocytochrome c and hydrogen peroxide was tried. The mechanism was represented as

$$Fe^{2+}cyt\underline{c} + H_2O_2 \xrightarrow{k_{+1}} Fe^{3+}cyt\underline{c} + \text{products}$$

$$Fe^{2+}cyt\underline{c} + H_2O_2 \xrightleftharpoons{\text{fast}} (Fe^{2+}cyt\underline{c}\cdot H_2O_2)$$

$$(Fe^{2+}cyt\underline{c}\cdot H_2O_2) + Fe^{3+}cyt\underline{c} \xrightarrow{k_{+2}} 2\ Fe^{3+}cyt\underline{c} + \text{products}$$

Using steady state approximations the following rate equation was derived from the proposed mechanism

$$-\frac{d[Fe^{2+}cyt\underline{c}]}{dt} = (k'_{+1}+k'_{+2}[Fe^{2+}cyt\underline{c}_{tot}])[Fe^{2+}cyt\underline{c}] - k'_{+2}[Fe^{2+}cyt\underline{c}]^2$$

which on integrating gave the following relationship between $[Fe^{2+}cyt\underline{c}]$ and time

$$t = \frac{2.303}{(k'_{+1}+k'_{+2}[Fe^{2+}cyt\underline{c}_{tot}])} \log \frac{\frac{k'_{+1}}{k'_{+2}} + [Fe^{3+}cyt\underline{c}]}{[Fe^{2+}cyt\underline{c}]} + C$$

This equation produces calculated curves similar to those

of (a) and therefore suffers from the same disadvantages.

The proposal of Shashoua[24] that the reaction might involve the combination of ferrocytochrome c and ferricytochrome c to form an active dimer was considered here. The mechanism proposed was

$$Fe^{2+}cyt\underline{c} + H_2O_2 \xrightarrow{k_{+1}} Fe^{3+}cyt\underline{c} + \text{products}$$

$$Fe^{2+}cyt\underline{c} + Fe^{3+}cyt\underline{c} \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} (Fe^{2+}cyt\underline{c} \cdot Fe^{3+}cyt\underline{c})$$

$$(Fe^{2+}cyt\underline{c} \cdot Fe^{3+}cyt\underline{c}) + H_2O_2 \xrightarrow{k_{+3}} 2Fe^{3+}cyt\underline{c} + \text{products}$$

which, after steady state approximations, gave the rate equation

$$-\frac{d[Fe^{2+}cyt\underline{c}]}{dt} = k_{+1}\,[Fe^{2+}cyt\underline{c}]\,[H_2O_2] + k_{+2}\,[Fe^{2+}cyt\underline{c}]\,[Fe^{3+}cyt\underline{c}]\left(\frac{k_{-2} + k_{+3} + k_{+3}\,[H_2O_2]}{k_{-2} + k_{+3}\,[H_2O_2]}\right)$$

On integrating this equation a relationship between $[Fe^{2+}cyt\underline{c}]$ and time is obtained

$$t = \frac{2.303}{k_{+1}\,[H_2O_2] + k_{+2}\,[Fe^{2+}cyt c_{tot}]} \log \frac{\frac{k_{+1}\,[H_2O_2]}{k_{+2}\,K} + [Fe^{3+}cyt\underline{c}]}{[Fe^{2+}cyt\underline{c}]} + C$$

where $K = \left(\frac{k_{-2} + k_{+3} + k_{+3}\,[H_2O_2]}{k_{-2} + k_{+3}\,[H_2O_2]}\right)$

This equation is similar to those obtained from the previous two mechanisms and produces a similar plot of $[Fe^{2+}cyt\underline{c}]$ against time, having good agreement with the experimental reaction curves in the intermediate range, but deviating at the extremes.

All these mechanisms, whose integrated rate equations contain a term of the form

$$\log \frac{[Fe^{3+}cyt\underline{c}]}{[Fe^{2+}cyt\underline{c}]}$$

all produce similar curves, which have deviations from the experimental reaction curves at the extremes.

(c) The previous mechanisms involved binding of ferrocytochrome _c_ with either hydrogen peroxide or ferricytochrome _c_. However it is more likely that the ferricytochrome _c_ will be capable of binding hydrogen peroxide, to form an active intermediate. A mechanism is investigated here which involves such an interaction. The mechanism considers direct interaction between ferrocytochrome _c_ and hydrogen peroxide and also the formation of an intermediate complex between ferricytochrome _c_ and hydrogen peroxide which reacts with ferrocytochrome _c_ The reaction sequence is as follows

$$Fe^{2+}cyt\underline{c} + H_2O_2 \xrightarrow{k_{+1}} Fe^{3+}cyt\underline{c} + \text{products}$$

$$Fe^{3+}cyt\underline{c} + H_2O_2 \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} (Fe^{3+}cyt\underline{c}\cdot H_2O_2)$$

$$(Fe^{3+}cyt\underline{c}\cdot H_2O_2) + Fe^{2+}cyt\underline{c} \xrightarrow{k_{+3}} 2\ Fe^{3+}cyt\underline{c} + \text{products}$$

Applying steady state approximations to this reaction sequence a rate equation is derived, incorporating the hydrogen peroxide concentration which is constant, into the rate constants k_{+1} and k_{+2}.

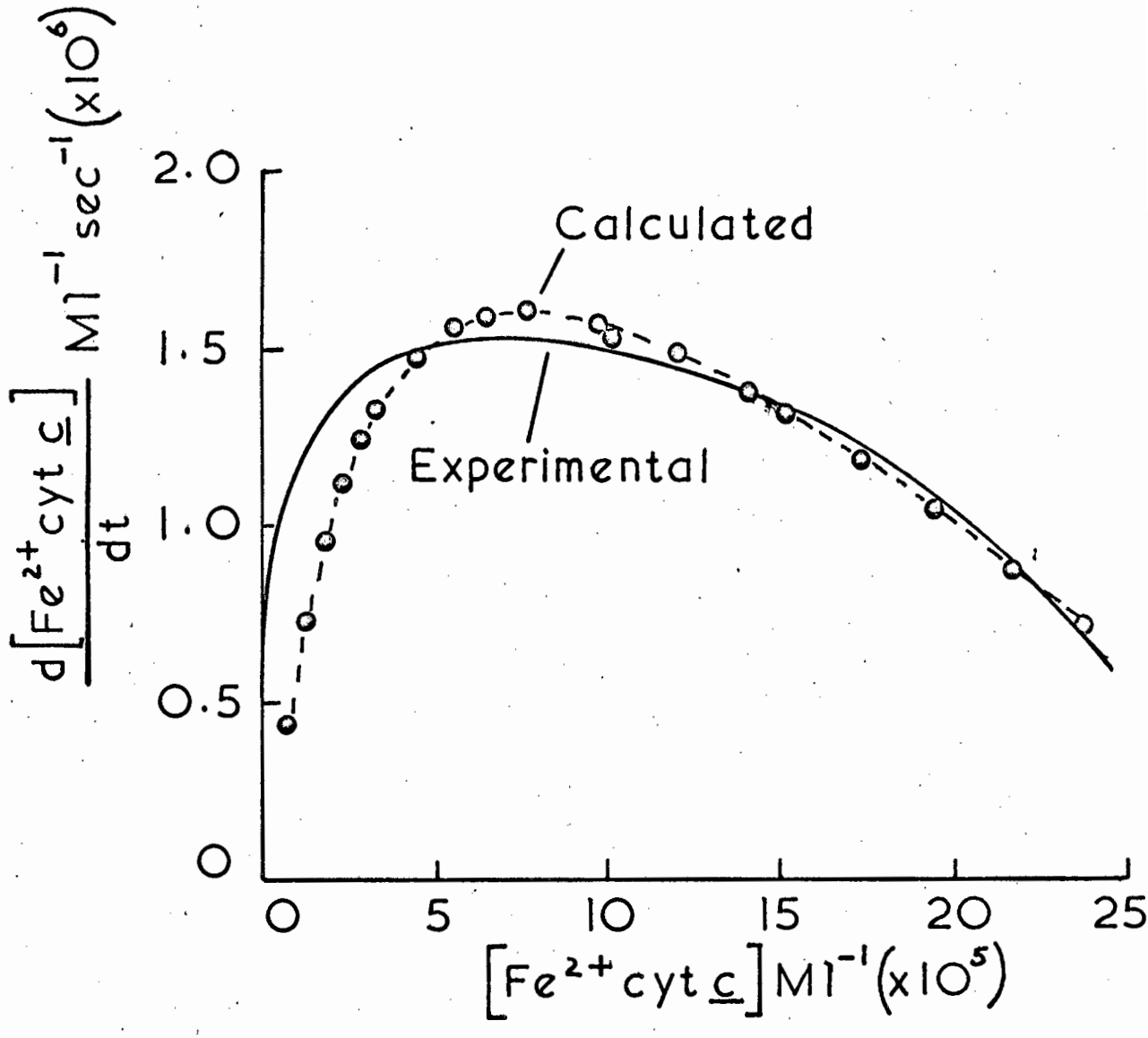
$$-\frac{d[Fe^{2+}cyt\underline{c}]}{dt} = \frac{\left(k'_{+1} + \frac{k'_{+2}k_{+3}}{k_{-2}}[Fe^{2+}cyt\underline{c}_{tot}]\right)[Fe^{2+}cyt\underline{c}] + \left(\frac{k'_{+1}\,k_{+3}}{k_{-2}} - \frac{k'_{+2}\,k_{+3}}{k_{-2}}\right)[Fe^{2+}cyt\underline{c}]^2}{1 + \frac{k_{+3}}{k_{-2}}[Fe^{2+}cyt\underline{c}]}$$

Since this equation is complex it was not integrated to give the relationship between $[Fe^{2+}cyt\underline{c}]$ and time. Instead the experimental reaction curves were used to obtain a curve of $-\frac{d[Fe^{2+}cyt\underline{c}]}{dt}$ against $[Fe^{2+}cyt\underline{c}]$ and this was used to test the rate equation derived from the proposed mechanism. The rate equation as stated above is in the form of a hyperbolic function

$$Y = \frac{a\,X + bX^2}{1 + cX}$$

where Y is the rate of the reaction, X is the ferrocytochrome c concentration and a, b and c represent the terms containing the combinations of rate constants. Values for the rate of reaction at different ferrocytochrome c concentrations were measured from an experimental reaction curve and then by numerical regression the best fit of the above equation was made to obtain values for the constants a, b and c. The values of a, b and c obtained, were used together with values for the ferrocytochrome concentrations to calculate the corresponding rates of reaction. These calculated curves of rate of reaction against ferrocytochrome c concentration were plotted and compared with the experimentally obtained curves (see fig. 29). The calculated curves corresponded closely to the experimental curve except in the region of low ferrocytochrome c concentration, representing approximately the last quarter of the reaction. In this region deviations occur between the calculated and experimental curves.

If the second step in the reaction sequence is considered to be irreversible, steady state kinetics can no longer be applied, but it is likely that the mechanism might give a better fit. The derivation of the rate equation for this mechanism involves the solution of three differential equations with respect to


$\frac{d[Fe^{2+}\ cyt\ c]}{dt}\ M1^{-1}\ sec^{-1}\ (x10^6)$
2.0
1.5
1.0
0.5
0
Calculated
Experimental
0
5
10
15
20
25
$[Fe^{2+}\ cyt\ c]\ M1^{-1}\ (x10^5)$

time, and difficulty is experienced in obtaining numerical solutions. Also, the mechanism predicts that at the completion of the reaction all the ferricytochrome c should be complexed to the hydrogen peroxide and no evidence was found to support this.

Although none of the mechanisms discussed here gave complete correlation with the experimental results, they give an indication of the complexity of the system involving product activation. It is likely that mechanisms which do not involve steady state approximations might give better correlation, particularly at the extremities of the reaction, where steady state approximations are least likely to apply.

The comparison of this reaction with that at pH 8.1 indicates a surprising increase in complexity resulting from the ionization of the cytochrome c molecule. Possibly a different mechanism is operative, or alternatively, steps subsequent to what was previously the rate determining step may become kinetically significant at lower pH values. Finally, it is difficult to relate the reaction studied here with that studied by Shashoua[24] at pH 7 where he observed two consecutive first order reactions, of different rates taking place during a single reaction. Evidently different experimental conditions, such as buffer

strength or reactant concentration, are responsible for the differences between the two reactions.

(3) The Reaction Catalysed by Copper.

The kinetic studies of the reaction between ferrocytochrome c and hydrogen peroxide in the presence of copper ions at pH 8.7 indicate that the rate has a simple first order dependence on each of ferrocytochrome c, hydrogen peroxide and copper ions. A simple rate equation which follows from this result is

$$-\frac{d[Fe^{2+}cyt\underline{c}]}{dt} = k\,[Fe^{2+}cyt\underline{c}]\,[H_2O_2]\,[Cu^{2+}]$$

Among reaction mechanisms which are consistent with this simple rate equation is

$$Cu^{2+} + Fe^{2+}cyt\underline{c} \underset{\text{fast}}{\rightleftharpoons} Cu^{+} + Fe^{3+}cyt\underline{c}$$

$$Cu^{+} + H_2O_2 \xrightarrow{k_{+2}} Cu^{2+} + \text{products}$$

which is analogous to that proposed for the autoxidation of ascorbic acid in the presence of copper ions[45,46]. The mechanism does not involve the formation of kinetically significant intermediate complexes, and these were also not suggested by either spectral evidence or saturation effects for the copper ions or cytochrome c, which might be expected if complex formation were an important part of the reaction. Although a simple

mechanism satisfies the observed kinetics for the copper catalysed reaction, this is an oversimplification of the reaction. The plot of the experimental rate constant k against Fe^{2+}cyt<u>c</u> (see fig.19) has a negative slope, suggesting that product inhibition of the reaction probably occurs.

Of particular interest in the study of the copper catalysed reaction was the rereduction which followed the oxidation of ferrocytochrome <u>c</u>. No detailed study of this reaction was carried out and therefore no attempt was made to fit any mechanisms to the reaction. Preliminary studies showed that the rereduction required the simultaneous presence of ferricytochrome <u>c</u>, hydrogen peroxide, and copper in the reaction mixture. It is interesting to consider what could occur in this reaction, especially since the reduced cytochrome <u>c</u> being formed does not undergo immediate re-oxidation. Conceivably the cytochrome <u>c</u> might be modified during the reduction, but this was not suggested by the spectral evidence and after the reaction was completed the cytochrome <u>c</u> could be fully reduced by sodium dithionite.

Boeri[47] etal have observed that ferricytochrome <u>c</u> in weak alkaline solutions becomes reduced after several hours in the absence of oxygen and Keilin and Hartree[48]

have observed that ferricytochrome c is slowly reduced by dilute hydrogen peroxide in weakly alkaline phosphate buffer, i.e.

$$2Fe^{3+}cyt\underline{c} + H_2O_2 \rightleftharpoons 2Fe^{2+}cyt\underline{c} + O_2 + 2H^+$$

It is possible that the copper is capable of catalysing this reaction and that this is the reduction reaction observed. In the reactions at pH 8.1 and pH 6.6 however, the reaction proceeded until complete oxidation of the ferrocytochrome c had occurred. The presence of copper might change the redox potential of one of the reagents sufficiently for a new position of equilibrium to occur, with about 60 - 70% of the cytochrome c in the reduced state. Assuming this, it is then surprising that the oxidation of cytochrome c proceeds beyond the equilibrium position (virtually to completion) and then returns to the equilibrium position. Evidently the change in redox potential caused by interaction of the reagents does not occur effectively until after the ferrocytochrome c has completely oxidized. An alternative explanation involves an oscillating system, such as described by Chance and co-workers[49], but the time scale of the events renders this unlikely.

Further investigation of this phenomenon is of some interest, but it was considered to be beyond the scope of this thesis.

V. CONCLUSIONS.

The discussion of the results obtained from this study enables several conclusions to be drawn about the reaction between ferrocytochrome c and hydrogen peroxide.

The hydrogen ion concentration was found to influence the reaction, an increase in pH causing a decrease in the rate of reaction. The change of reactivity of the cytochrome c between pH 7.6 and pH 7.9 is attributed to the effect of pH on the reaction. It is probably caused by an ionization taking place in the cytochrome c molecule, the most likely ionization being the dissociation of a hydrogen ion, mentioned by Rodkey and Ball[17].

An increase in the ionic strength of the solution causes a small increase in the rate of reaction. The net charge created in the formation of the transition state is only + 0.5, indicating that not much charge is formed before the rate determining step of the reaction.

The rate of the reaction was found to increase with increasing temperature, at pH 8.1, pH 6.6 and at pH 8.7 in the presence of copper ions. The activation energies, enthalpies of activation and entropies of activation were calculated and are summarized in Table 4 (page 71).

The kinetics of the reaction between ferrocytochrome c and hydrogen peroxide were studied at pH 8.1, pH 6.6

and at pH 8.7 in the presence of copper ions. At pH 8.1 the kinetics were found to be simple, the rate of reaction having a first order dependence on ferrocytochrome c and hydrogen peroxide. The simple rate equation

$$-\frac{d\,[Fe^{2+}cyt\underline{c}]}{dt} = k\,[Fe^{2+}cyt\underline{c}]\,[H_2O_2]$$

was proposed and the rate constant for the reaction was found to be of the order of 5.5 $l.mole^{-1}sec.^{-1}$.

The kinetics of the reaction at pH 6.6 gave a simple first order dependence of rate on both ferrocytochrome c and hydrogen peroxide. Product activation was found to occur at this pH, the ferricytochrome c which was formed during the reaction, acting as a catalyst. A rate equation could not be found which accounted for the catalytic effect of ferricytochrome c, and fitted the observed kinetics. The experimental rate constant for the reaction was found to be of the order of 19.5 $l.mole^{-1}$ $sec.^{-1}$.

A copper - histidine complex was found to catalyse the oxidation of ferrocytochrome c by hydrogen peroxide at pH 8.7. The kinetics of the reaction showed a simple first order dependence on all three reactants and a simple rate equation was proposed

$$-\frac{d[Fe^{2+}cyt\underline{c}]}{dt} = k[Fe^{2+}cyt\underline{c}][H_2O_2][Cu^{2+}]$$

The rate constant for this reaction was measured and found to be of the order of 5×10^4 $l^2 mole^{-2} sec.^{-1}$. The reaction involving copper catalysis was unusual as the ferrocytochrome _c_, after complete oxidation, was found to undergo reduction, which was dependent on the simultaneous presence of both copper and hydrogen peroxide. However insufficient experimental detail precluded a detailed analysis of this reaction.

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THE GEOCHEMICAL DISTRIBUTION OF SOME ELEMENTS IN ULTRABASIC ROCKS

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L.G.H.

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All maps, tables and diagrams will be found in the Appendix.

ABSTRACT

A suite of 228 ultrabasic rocks has been studied, of which, 101 peridotites and 23 eclogites are kimberlite xenoliths, and 73 are kimberlites.

Determinations of K, Li, Rb, Cs, Zn, Th and U in the ultrabasic material are presented. The analyses have been carried out using carefully standardised instrumental methods.

An optical spectrographic procedure for the determination of Rb, Cs and Li simultaneously in ultrabasic rocks has been developed. Detection limits of < 0.1ppm. have been obtained for Rb and Cs.

Evidence is presented to show that most nodules in kimberlite have been contaminated. K, Rb, Cs, Li and some Zn contamination is reported. Many trace element studies on the nodules have limited significance, as a result of this contamination.

Very constant zinc contents have been found in peridotites, and it is suggested that the zinc content of a garnet peridotite mantle would be close to 34ppm.

Thorium and Uranium determinations in kimberlites are reported.

It is suggested that minor mineral phases, including phlogopite, are probably present in the mantle. A potassium rich phase may be essential to the apparently low temperature emplacement of kimberlite.

The association of diamond and eclogite is discussed.

High K/Cs ratios are reported for olivine melilitite, the highest ratio being 47,000.

CHAPTER ONE

I N T R O D U C T I O N

There is much current interest in the chemical composition of ultramafic rocks. This is due in part to the fact that geophysical, geochemical and petrological arguments have led many workers to the conclusion that the mantle is ultramafic in character, and probably closely corresponds to a four phase spinel or garnet peridotite in composition. The actual composition can best be estimated by the study of rocks of supposed sub-crustal origins. Such material is represented in many alkali olivine basalts by nodules of peridotite, garnet peridotite and eclogite and examples are known of inclusions in tholeitic basalts. Kimberlite diatremes, too, contain xenoliths, many of which are ultramafic in character, and it is these which are the primary object of study in this thesis.

The mantle comprises 68.1% of the weight and 83% of the volume of the earth. In addition to its sheer bulk, it is important because of the role it has had to play in the differentiation history of the earth, and the evolution of the crust as it is today. There is abundant evidence that basalt magma generation has its origins in the upper mantle. Indeed, the crust of the earth is so relatively thin (~35km. in continental areas and~8km. in oceanic areas) that explanations of most of the major geological features of the earth's crust

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must take into account some influence derived from the mantle.

Here it is necessary to outline the observations and ideas which have led to the conclusion that the ultramafic xenoliths found in kimberlites are likely to have been transported from the region of the upper mantle. There are currently four independent methods of obtaining an estimate of the composition of the mantle. These are seismology, heat flow, petrology, (field and experimental) and geochemistry (cosmic and terrestrial).

The number of rock compositions that have seismic velocities equal to those observed for the upper mantle is limited to ultramafic and eclogitic compositions (BIRCH, 1960). Further, it has been shown, (BIRCH, 1964) that ordinary dunites, bronzitites, diabases and eclogites with 8-10% FeO roughly follow the expected density distribution in the lower mantle.

Empirical observation of the earth's surface heat flow indicates a rough equality for the heat flow in the oceanic and continental areas, which in turn implies a difference in the potassium, thorium and uranium content of the sub-continental and sub-oceanic mantle (BIRCH, 1964). The more differentiated sub-continental mantle is probably also more impoverished in the low melting silicates rich in Na_2O, K_2O, CaO, FeO, Al_2O_3 and H_2O. However, the absolute heat flow values tell us little about the major element chemistry of the mantle, for these major elements are non-radiogenic.

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The petrological approach limits the composition of the upper mantle to lie between a basalt and a peridotite, primarily within the five component system $MgO-CaO-FeO-Al_2O_3-SiO_2$. Other components such as Cr_2O_3, Na_2O, K_2O, and TiO_2 will be present in lesser quantities and generally these constituents have been treated as dilute solutes in phases already present, namely forsteritic olivine, enstatite solid solution, diopside solid solution and garnet or spinel. However, small amounts of such minerals as phlogopite or ilmenite may also occur.

O'HARA (1967), has devised a petrogenetic grid making use of the chemical compositions of clinopyroxenes coexisting with olivine and orthopyroxene. The results of this confirm the apparent derivation of nodules in kimberlite from an environment consistent with equilibration at depths of 100 to 140km. on the Precambrian shield geothermal gradient. These nodules appear to have been transported from greater depths than any other material available at the earth's surface for study.

Assuming that the chondritic meteorites are representative of average cosmic material, UREY (1952), McDONALD (1959) and RINGWOOD (1959), amongst others, have proposed a carbonaceous chondrite composition as a reasonable model for the composition of the earth. Some aspects of heat flow evidence support the proposal but limitations are imposed by the comparative distribution of certain trace elements in the crust and

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upper mantle of the earth, GAST (1960). RINGWOOD (1966) has compared the composition of the whole mantle from a chondritic model with a composition for the upper mantle calculated from his "pyrolite" model, an arbitrary mixture of three parts dunite and one part basalt. Whilst concluding that it was possible that the earth had retained the primordial abundances of elements which are not readily volatile under high temperature reducing conditions, it appeared in contrast, as though the earth had suffered strong depletion in a large number of volatile elements such as sodium, potassium, rubidium, cesium, zinc, cadmium, bismuth, silver, thallium and lead.

These conclusions were drawn from data for the relative abundances of volatile elements in the upper mantle and in Type I carbonaceous chondrites. It was pointed out that this data was both sparse and of poor quality.

Throughout the present investigations, in which the geochemical distribution of a number of elements, including some of these same volatile elements, have been studied, the author has been mindful of the fact that the need is for data on ultramafic material which is precise and accurate. Trace element data for ultramafic rocks was reviewed by TUREKIAN and WEDEPOHL (1961) and VINOGRADOV (1962). Much of this information was obtained using spectrochemical techniques, often working near the sensitivity limit, and is of doubtful signi-

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ficance. Sinch then, additional data has been published, much of it determined by more accurate and precise methods, such as isotope dilution or neutron activation analysis.

A more recent review (GOLES, 1967), whilst revising abundance data for 26 elements in ultramafic rocks in general, emphasises the paucity of reliable trace element analyses in ultramafic inclusions in kimberlite, and the doubtful significance of an average value obtained for all ultramafic rock types grouped together.

Potassium is an example of an element for which improved methods of analysis at low concentration levels have recently been developed, and applied. An indication of the unreliability of much of the older data published on this element is contained in Table I.

Until quite recently the majority of potassium analyses were carried out using the wet chemical J. LAWRENCE SMITH procedure. The results were generally systematically low, due to occlusion of potassium on precipitates and to loss of potassium, when volatilising ammonium salts. However, at low concentration levels of alkali, the method appears to yield high results, possibly due to the presence of a blank. (AHRENS PINSON & KEARNS, 1952). It is clearly shown in Table I that the older data, extracted from WILLIAMS (1932) is unsatisfactory, both for sodium and potassium, a conclusion also reached

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CHAPTER TWO

SAMPLES AVAILABLE FOR ANALYSIS AND THEIR PREPARATION

Samples available for analysis.

These comprised a total of one hundred and one peridotites, twenty three eclogites and seventy three kimberlites. They represent material from eleven South African occurrences and one in Tanzania.

Thirty eight peridotites and thirteen kimberlites are from the Bultfontein Mine. The samples from Wesselton, De Beers, and Du Toit's Pan bring the number of samples from the major group of kimberlites in the Kimberley area to one hundred and fifteen. The collection therefore contains samples from a geographically large area and a comprehensive suite of samples from one locality.

It had been recognised that any study of the xenoliths, and particularly of their alkali metal content, would be invalid without taking into account the influence of the host kimberlite, which has a high potassium content and a high potassium to sodium ratio. Wherever possible the kimberlites, from which nodules were obtained, were sampled. Both nodules and kimberlite are available from Bultfontein, Du Toit's Pan, De Beers, Wesselton, Jagersfontein and Roberts Victor Mines.

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Many specimens were provided from the Williams Collection described by WILLIAMS (1932). These were augmented by G.W. BERG, who undertook a field trip with the full assistance of the Geological Section of Anglo-American Corporation in Kimberley. Other samples were obtained as opportunity presented itself. In addition samples of related ultrabasic materials were obtained.

A total of two hundred and twenty eight rock specimens were available for analysis. The sources of the material have been acknowledged at the end of the thesis.

Sample Classification

In this study the petrographic description of the samples is brief. It will be covered in greater detail by G.W. BERG and J.C. SIEBERT. SIEBERT will be submitting a study of the mineralogy and petrology of much of this material in fulfilment of the requirements of an M.Sc. degree at the University of Cape Town.

It was felt that a relatively simple approach would suffice in this study, in which only whole rock analyses are presented. Details of the samples, their locality of occurrence, classification and size are contained in the Appendix (Section A).

Modal analyses of the four major primary phases, olivine, enstatite, garnet and diopside in many of the nodules, have kindly been made available by J.C. SIEBERT, and are included in Table A 1.

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The samples designated eclogite are all essentially garnet and omphacitic pyroxene assemblages. One has accessory corundum, and another accessory kyanite. All have garnet modes greater than 30%. None have olivine as a constituent.

Samples in which two pyroxenes appear and constitute more than 80% of the rock, have been designated pyroxenites, whilst the rest of the xenoliths all fall into a group in which olivine predominates over the two pyroxenes, and garnet is always less than 20% of the whole. These peridotites have been assigned to one of the four subdivisions:-
lherzolite, garnet lherzolite, harzburgite and garnet harzburgite. No dunites were available (olivine $>$ 90%). No attempt has been made to classify the kimberlites, which is in any case, very difficult.

Sample Preparation

It was the intention that, ultimately, a detailed study of the samples would be undertaken, far beyond the scope of the present investigation. A scheme was drawn up which would provide sufficient material for a complete geochemical investigation. This would include a geochemical study of the individual minerals of the xenoliths, as well as of the sample as a whole.

It was recognised that there were serious sampling problems to overcome. The xenoliths are coarsely crystalline, and the minor mineral phases are irregularly distributed. In the

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eclogites, banded samples are known, whilst individual portions of a sample are often richer in one of the two principal phases than the average for that sample. In both eclogite and peridotite, the accessory minerals may be scattered irregularly throughout the rock, or may be concentrated in a particular part of it. This was particularly true of the phlogopite mica, which was expected to account for a considerable proportion of the potassium content of some of the xenoliths. Study of a thin section from one peridotite sample indicated the presence of mica to the extent of approximately 10% of the whole. Two other thin sections, from different portions of the same sample, were practically devoid of the mineral, which was seen in hand specimen to occur as occasional irregular blebs throughout the rock.

Furthermore, many of the xenoliths had suffered secondary alteration to their exterior surfaces. This could be due to the effect of the kimberlite matrix and its related fluids, or to secondary alteration due to oxidation at or near the surface. Many of the xenoliths analysed here were collected from the mine waste dumps, where they may have lain for as long as 60 years. Many more were probably originally found in the zone of secondary alteration of the kimberlite, which often extends to depths of many tens of feet below the surface of an outcrop. Finally, the xenoliths are of a finite size, and whilst large samples weighing up to 50kg. have been seen, the average size is much less.

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The problems of sampling kimberlite in a representative manner, are even greater than those for the nodules. However, with kimberlite, there is only a practical limitation on the size of a sample collected, which is a compensation. In hand specimen kimberlite has a porphiritic texture, and contains large commonly rounded crystals and nodules of olivine, enstatite, phlogopite mica, chrome diopside, garnet and ilmenite embedded in a serpentinous ground mass. Whilst the "cognate" inclusions, thought to be derived from the deeper layers of the earth are scarce, accidental inclusions are common. These consist of representatives of the surrounding country rocks; mainly basalts, shales, sandstones and mudstones in the Kimberley region, with occasional granite and gneiss, brought up from the basement complex of that area. These accidental inclusions are clearly not a part of the kimberlite rock itself. Geochemically the two are unrelated to each other, yet it is often not possible to obtain samples of kimberlite which do not contain small inclusions of the accidental type.

Kimberlite weathers easily, and this occurs in the form of chloritisation and carbonatisation of the basic rock-forming minerals. Carbonates of calcium and magnesium, derived from the breakdown of silicates, impregnate the mass, which in its ultimate form is a soft, yellowish, speckled mass, known as "yellow ground". Partly weathered, dark-coloured kimberlite, is known as "blue ground". At depth, beyond the influence of supergene weathering,

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the blue ground passes gradually into kimberlite rock.

A sample preparation scheme was evolved, which made the best possible use of the material available. A complete record was kept, in which the whole procedure, as applied to each sample, was noted. Included in this were details of the initial size of the sample, the amount crushed for mineral separation work, and the amount crushed for whole-rock analysis. Also included were details of the crushing apparatus used, as this had a bearing on the problem of contamination.

As can be seen from Table A 1`, only large kimberlite samples were prepared. Large peridotites and eclogite samples were studied, when available, but in order to have a representative selection, it was necessary to include smaller samples. It may be relevant to consider the sample size when assessing the data presented later.

When crushing a kimberlite, a minimum of 1.0kg. was broken into pieces in a vice and taken down to $\frac{1}{4}$ inch chips in a carbon steel rotary jaw crusher. This amount was quartered, and then ground finer to -40 mesh in a steel ball mill, with a rotatory grinding motion. This quartering was repeated, and approximately 130gm. taken down to -120 mesh, in the same vessel. Any small residue not ground sufficiently finely, was reduced to the required size in an agate mechanical grinder, and the fine material carefully re-mixed. A hand specimen of the sample was always

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retained, and this and any other material was carefully packed and stored. Thin sections were prepared where possible. Samples containing large, obviously accidental xenoliths, were avoided.

The procedure adopted for peridotites and eclogites was slightly different. First, the exterior surface was sliced off, leaving an approximately rectangular block of fresh material, which was originally the centre of the usually rounded nodule. Thin sections were then made. The number depended on the importance of the sample, and was at the discretion of G.W. BERG. After washing in acetone to remove the oil from the diamond saw and scrubbing clean in de-ionised water, the nodule was dried at 120°C. The rectangular block was then broken into smaller pieces of approximately 25gm. each, and 500gm. (total weight) of these were selected randomly. This amount was reduced to $\frac{1}{4}$ inch chips in the carbon steel rotary jaw crusher, and reduced to -40 mesh in the ball mill. Upon quartering approximately 130gm. was reduced to -120 mesh, in the same vessel. Any small quantity of material, found to be over-sized, was fine ground in a mechanical mortar machine, using an agate grinding vessel.

When a sample weighed less than 500gm., the whole sample, except for one small hand specimen, was crushed. Suitable proportions of -40 mesh and -120 mesh material were obtained by the method outlined above. The -40 mesh material was intended for mineral separation work.

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Handling of the material was avoided wherever possible, and the powdered samples were well mixed after crushing. The powders were stored in clean glass bottles, and these were shaken vigorously before any of the contents were removed. The crushing apparatus was kept scrupulously clean and every effort was made to avoid contamination of any sort.

No evidence of contamination has been found for any of the elements studied in this thesis. Iron contamination was detected, but does not affect the data sought here.

Certain samples, amongst them the eclogites and those peridotites which formed part of the Williams Collection, were crushed early in the project, before the mechanical ball mill or the rotary jaw crusher were available. In terms of quantity, the same scheme outlined here applies to these samples, but the material was crushed in a hand mortar and pestle, also made of carbon steel.

The whole of the section of the work relating to sample preparation, was undertaken as a joint project with G.W. BERG. The contamination aspect concerns elements studied in his thesis, and will be dealt with there.

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CHAPTER THREE

A SURVEY OF THE POSSIBILITY OF USING COMBINED ION-EXCHANGE ENRICHMENT AND OPTICAL SPECTROGRAPHIC TECHNIQUES IN THE ANALYSIS OF TRACE ELEMENTS IN ULTRAMAFIC SILICATE ROCKS.

In this chapter, the results of a survey on the suitability of ion-exchange pre-enrichment methods, as an aid to subsequent spectrographic analysis of ultramafic rocks, are reported. The initial work was a logical extension of that carried out by BROOKS (1960) and EDGE (1960) and reported in a series of publications which appeared in Geochimica et Cosmochimica Acta in the period 1959 to 1961. These papers formed the basis of an analytical scheme, details of which have been described by AHRENS, EDGE and BROOKS (1963).

It may be recalled from the Introduction that the volatile elements, potassium, rubidium, cesium, bismuth, cadmium, tin, lead, zinc, thallium and silver, were of interest. Considering the trace elements only, and not potassium, it was shown experimentally that existing optical spectrographic methods were only sensitive enough for the determination of zinc in most samples of ultramafic rocks. This was in agreement with the information derived from the abundance data of TUREKIAN and WEDEPOHL (1961) and VINOGRADOV (1962), when taken in conjunction with the quoted detection limits of D.C. arc spectrographic procedures (AHRENS and TAYLOR, 1961). As the D.C. arc spectrographic procedure was the most sensitive analytical technique available to the author, it

is pertinent to consider the enrichment factors which would apparently be required for the determination of this suite of elements. Table 3 summarises the relevant information. It should be noticed that the enrichment factors necessary for bismuth and cadmium determinations are extremely high, and their determination would necessitate using large initial amounts of sample in any pre-enrichment technique.

These techniques are discussed in two sections concerned with cation and anion exchange respectively.

A. Cationic Ion-exchange Procedures.

R.A. EDGE (1960) has developed an ion-exchange enrichment technique combined with optical spectrographic analysis for trace constituents in common silicate rocks. In particular rubidium, cesium and the rare earths have been studied, and a procedure for analysis proposed. Furthermore a modified procedure, specifically designed for basic rocks, and an even more sensitive method, which had been applied to chondrites were available from this work.

Although a great amount of information was available in the literature on ion-exchange separations of the alkali metals, no other application of these techniques to the estimation of rubidium and cesium in silicates was known. The procedure recommended by EDGE (AHRENS, EDGE and BROOKS, 1960) for basic rocks was considered, therefore, to be the best starting point in this investigation. However, as the method was found to be unsuitable for this author's

purposes, it is not proposed to describe it in detail. Instead, a brief outline will be given, together with the results obtained, and the reasons for not adopting the procedure for further determinations.

In the proposed method, the ultramafic samples were first attacked and decomposed with hydrofluoric and perchloric acids in platinum vessels. After evaporation the residue was taken up in 20 ml of 2N HCl, and soaked into a column of Dowex 50 ion-exchange resin. Elution with 260 ml 3N HCl was commenced at a flow rate of 20 ml/hr. The eluant fraction from 160-260 ml was expected to contain the potassium, rubidium and cesium fraction from the sample, together with calcium. However, as will be discussed later, this was not always the case.

A second column elution, using 180 ml 12N hydrochloric acid as eluant at a flow rate of 25 ml/hr was required to remove the alkali metals whilst leaving the calcium behind. After evaporation of this second eluant fraction to dryness and treatment to ensure a common matrix, the residue was loaded into graphite electrodes and arced at 5 Amps on a Hilger Spectrograph. (Glass optics).

This method for basic rocks was applicable to the determination of rubidium and cesium in eclogites without alteration. It was not suitable for the determinations in peridotite or kimberlite, however. This was due to the extremely high concentration

of magnesium in the latter, with which the column chromatographic procedure was unable to cope. As illustrated in Figure 1, magnesium's position in the elution sequence in HCl on Dowex 50 resin, was just ahead of the alkali elements, potassium, rubidium and cesium. In the case of high magnesium rocks, the magnesium and the alkalis were eluted together and in a greater volume of effluent. This effect is not unexpected, and according to theoretical considerations increasing the aspect ratio of the chromatographic column (i.e. the ratio of the length to the diameter) should improve any separation. However, although an improvement was obtained, it was not sufficient to offer any hope of successful cesium determinations for peridotites.

Ion-exchange separation and pre-concentration usually fall into one of three categories. The most favourable group is that where the matrix is not adsorbed on the ion exchanger at all. In such column operations, a large amount of sample solution can be introduced into a small column, having low exchange capacity, without fear of breakthrough of the desired elements. The adsorbed trace elements are then eluted from the column with a small amount of an appropriate eluant. The anion-exchange procedure of BROOKS (1960) falls into this category and is discussed later. The second type of process is the reverse of the first. Here the matrix is absorbed and the trace elements are not. The main consideration is the breakthrough of the matrix elements from the

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column, due to the saturation of the resin. The third group contains those methods in which both the trace and the matrix elements are adsorbed on the column.

The procedure dealt with here for determining rubidium and cesium falls into this third category. It is the most unfavourable case. Although separations can be carried out, it is generally found that careful manipulation is required to adsorb the elements quantitatively and separate them chromatographically. In these separations changes in the matrix radically affect the elution characteristics of the resin column, and this is exactly what occurred in this study. A peridotite contains approximately 40% MgO, whilst the geological material for which the method was designed contains much less. The magnesium chromatographic peak tailed excessively and a separation of the alkali metals, potassium, rubidium and cesium, from magnesium, could not be obtained.

The method suffered from further disadvantages. It was necessary to regenerate the columns with 6N HCl and to conduct one of the elution processes with 12N acid. This was physically unpleasant, and as the procedure takes a considerable time, leads to the building up of a corrosive atmosphere in the laboratory, unless the operation is carried out in a fume cupboard. Furthermore the resin was continually swelling and contracting, in response to the widely differing acid concentrations used (3N, 6N and 12N). This gives rise to constantly reducing flow rates,

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B. Anion-Exchange Enrichment Procedures

BROOKS (1960) developed a procedure, combining anion-exchange enrichment and spectrochemical analysis, for the quantitative determination of many trace elements including zinc, bismuth, tin, thallium and cadmium. Silver and lead were also enriched in the ion-exchange process. Using this technique, zinc, bismuth, tin, thallium and cadmium were determined quantitatively in a suite of twenty-nine rocks. Subsequently BROOKS and AHRENS (1961) estimated cadmium, thallium and bismuth in forty-four samples. Amongst the analysed materials were several ultramafic or ultrabasic specimens (Table 6).

A preliminary investigation was carried out to test the applicability of the procedure as a general analytical method for a large number of samples. The basis of the determination is the dissolution of the sample in hydrochloric acid and the sorption, onto an anion-exchange column of the chloro-complexes of the trace elements in the sample. The sample matrix is allowed to pass through the column, after which the trace elements are removed by elution. Fortunately not one of the major constituents of rocks is strongly adsorbed under the conditions used. The separation of iron from the trace elements is particularly advantageous as this element has a complex emission spectrum which can be a source of spectral interferences in optical spectrography. The method has the advantage that the enrichment factor can be

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increased at will, by simply dissolving more material and introducing it in solution on to the column.

The recommended procedure was followed in its entirety. Two peridotites (10gm.), two eclogites, (5gm.), a kimberlite (2gm.) and a synthetic "eclogite" containing no trace elements (2gm.) were dissolved in hydrofluoric, nitric and sulphuric acid mixtures in Teflon beakers. After evaporation, the soluble residue was taken up in hydrochloric acid solution and absorbed onto the anion-exchange column. After absorption, the column was washed with hydrochloric acid and eluted with nitric acid. 20mgm. of sodium chloride in acid solution was added to the eluant, to serve as a carrier. This liquid concentrate was evaporated to dryness, and the residue examined spectrographically. Reagent blanks were also examined in the same way at the same time.

Silver, tin, zinc, lead and thallium were detected in all samples; cadmium was detected in the kimberlite and the eclogites, but the positive identification of bismuth was obscured by interference on the most sensitive spectral line of bismuth. This was due to a series of lines in a weak band spectrum occurring in the bismuth lines' wavelength range. Silver, lead, zinc and tin were detected in the reagent blanks, and this was due to contamination. Although Analar reagents were used at all times, and despite the precautions taken in the operation of the method, and the manipulation of the apparatus, this contamination could

not be prevented. Zinc, for example was found to be present in the ion-exchange resin. The amount of contamination was equivalent to approximately 0.3ppm. of silver, 3ppm. of tin, 3ppm. zinc and more than 5ppm. of lead in a 2gm. sample of material.

The band structure which interferes with Bi 3067.7Å and which was mentioned previously, was present on every spectrogram taken during this preliminary study. The interference had previously been noted by C.O. OOSTHUIZEN and J.P. WILLIS (1964) whilst applying the same analytical technique to manganese nodules. They had assumed it to be an OH band without positively identifying it.

A positive identification was made. The band is sensitively emitted by a D.C. arc in the presence of water vapour, even if the concentration of the water vapour is very low (JACK, 1927).

An examination of a large number of spectrograms showed the presence of —OH band components in every case. The band is degraded towards the red and has a head at 3063.4Å.

Interference of the —OH band component with Bi 3067 may be illustrated by the enlargement shown in Plate 1 where self-reversed Bi 3067 is superimposed on to the —OH band spectrum; the interfering —OH component is clearly seen in the centre of the self-reversed line. On the original photographic plate a second line of the OH band could be seen towards the longer wavelength side of the gap of the self-reversed line and this will also interfere with the bismuth line. These two lines of the

PLATE 1

INTERFERENCE ON Bi 3067.7

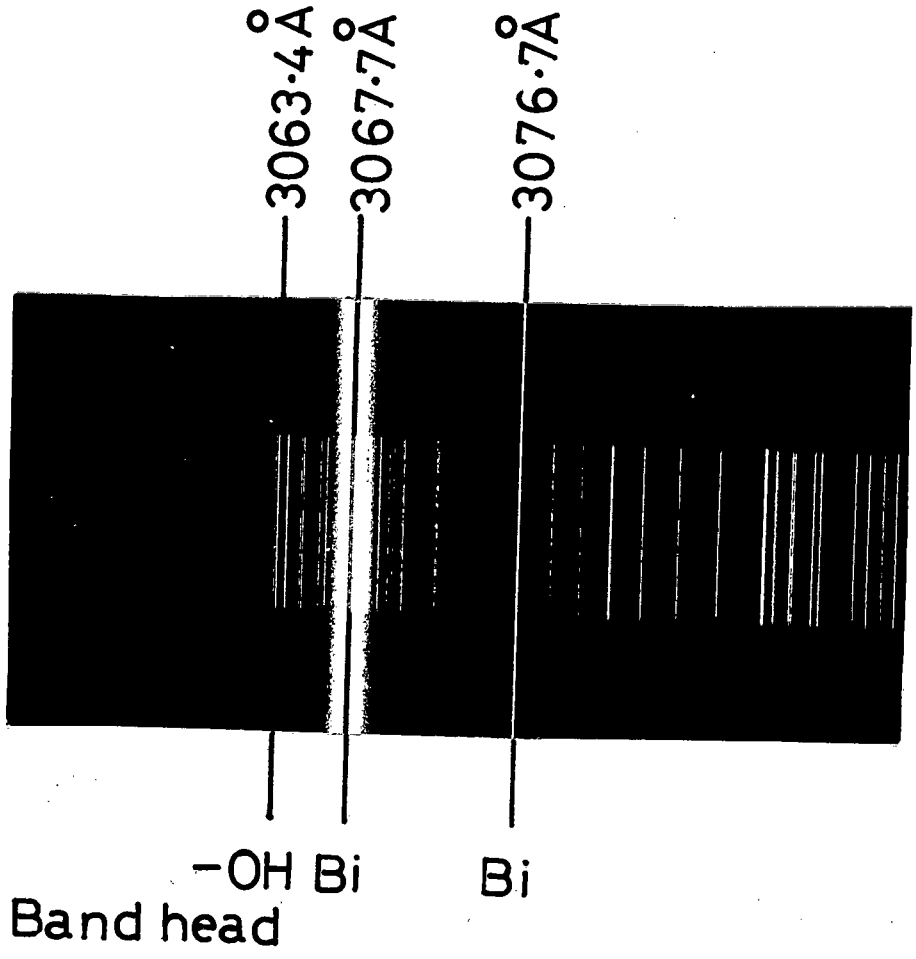

PLATE 1

Interference on Bi 3067·7

Magnification X 12

band are part of a triplet, the third member of which may cause additional interference.

Two sources of water vapour have been found, namely, the arc atmosphere and the electrodes plus sample. The first source is the major contributor. Further work has shown that drying the sample at 120^{0}C for four hours and arcing in a dry atmosphere removes all traces of the band, which is always present if these precautions are not taken. It has been found that a most convenient means of obtaining a dry arcing atmosphere is to use a Stallwood type jet and to obtain gas mixtures from cylinders. Use of dry argon or dry argon/oxygen atmospheres has the advantage that the bismuth line is enhanced under these conditions (INGAMELLS and SUHR (1963); MORRISON, RUPP and KLECAK (1960). Other dry atmospheres do not produce this latter effect.

Making use of pre-enrichment and a modified arcing technique, bismuth was detected in a kimberlite, but not in an eclogite, nor in two peridotites.

An assessment of the information obtained so far showed that this approach could well yield useful data on thallium and might also be applicable to the determination of bismuth and cadmium in certain samples. It could not be used for the determination of zinc, lead, silver or tin, due to the relatively large reagent blanks. One of the principal advantages of the original method had been the fact that a number of elements could be determined

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simultaneously. This now largely fell away. Furthermore, the enrichment factor required necessitated the use of at least 5gm. of material (in duplicate) for each peridotite and eclogite, and often more. As ultrabasic material is at a premium, and as it was unlikely that the data obtained would be significantly better than that already available, it was not felt desirable to proceed with quantitative determinations by this pre-enrichment spectrographic technique.

BROOKS (1960) did not perform full replicate analyses. His duplicate determinations referred to the spectrographic procedure only, and did not reflect any error which might arise from the column and dissolution procedures. AHRENS, WILLIS and OOSTHUIZEN (1967) who performed full replicate analyses, report relative standard deviations of about 20% for the method. This is not satisfactory, and confirms the experiences of this author in the determination of cesium in eclogites, by the pre-enrichment technique of EDGE (1960), which were reported earlier.

As a result of this reconnaissance study, it was the author's opinion that it was not justifiable to apply these methods to the analysis of ultramafic materials. Apart from the shortcomings of the methods themselves, it seemed likely that alternative approaches to the analytical problems could provide superior results.

The work of MORRISON, RUPP and KLECAK (1960), (1960a),

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JOENSUU and SUHR (1962) and INGAMELLS and SUHR (1963) had substantially lowered the detection limits of the elements lead, (0.3ppm.) bismuth, (0.1ppm.), silver (0.01ppm.), tin (0.3ppm.), zinc (0.1ppm.) and thallium (0.3ppm) in direct optical spectrographic determinations. These methods made use of atmospheres such as argon and argon/oxygen and relatively high temperature arcing conditions. They largely avoided contamination problems.

Using an argon/oxygen atmosphere and similar operating conditions, zinc was detected in peridotites, eclogites and kimberlites. Thallium was detected in kimberlites and some eclogites by a method in which lithium carbonate was mixed with the sample in a 1:3 ratio and the arcing was carried out at 3.5 Amps in an argon/oxygen atmosphere, using a Stallwood type jet.

The original application of the ion-exchange, pre-enrichment techniques to provide geochemical abundance data where this was either scanty or missing, was a valid one. It was this author's opinion, however, that such methods could not fulfil the rather more stringent requirements of the proposed study of trace element distributions in potential mantle materials. Accordingly this preliminary investigation was discontinued.

CHAPTER FOUR

THE DEVELOPMENT AND STANDARDISATION OF AN IMPROVED SPECTROGRAPHIC TECHNIQUE FOR THE DETERMINATION OF RUBIDIUM, CESIUM and LITHIUM

The determination of rubidium, lithium and cesium has often been carried out by optical spectrographic techniques. The operating conditions, analysis lines, internal standard lines and other parameters differ from method to method, according to the particular problems faced by each analyst. The literature prior to 1960 is reviewed by AHRENS (1961). The most recent alkali metal determinations using the optical spectrograph are those of MAKSIMOVIC (1967), who has estimated the amount of the elements sodium, potassium and lithium in Yugoslavian ultramafic materials.

The average level of concentration of the alkali metals in ultrabasic rocks is low. It was the aim of this study to develop a method which could be used for the determination of the three trace elements, lithium, rubidium and cesium simultaneously in these materials. The concentration ranges for which the method was required were:-

Li	1 - 20ppm.
Rb	0.1 - 25ppm.
Cs	0.1 - 10ppm.

Previously reported work using an optical spectrographic D.C. arc technique has usually been carried out using the glass

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optics of a prism spectrograph. The dispersion of a glass prism in the wavelength range of the spectrum in which the most sensitive alkali lines appear is extremely poor (~40Å /mm.). The conventional optical system also has other inherent defects. The net result is a procedure in which poor line shape, possible line interference, and bad background characteristics militate against obtaining accurate and precise results at low levels of concentration.

The detection limits quoted for instruments of this type are

Li ~ 0.5ppm.

Rb ~ 1.0ppm.

Cs ~ 2.0ppm.

(AHRENS and TAYLOR, 1961).

The quoted detection limit for Li would be satisfactory for our purposes were it not for the fact that the most sensitive line of lithium (6707Å) is liable to show self absorption at concentrations greater than 5ppm. Ca 6102.7 interferes with the next most sensitive lithium line at 6103.6 in a glass prism spectrograph. The only other lithium line in the red or infra-red region of the spectrum, Li 8126, is normally not sensitive enough for the purposes of this investigation.

The quoted detection limits for rubidium and cesium were clearly not satisfactory for our purposes.

AHRENS (1954) has developed a general method for the determination of potassium, lithium, rubidium and cesium in common rocks and minerals. The analysis lines used include K 6939 and 6911; Li 6103, 6707, and 8126; Rb 7800 and 7947; and Cs 8521Å. Sodium is used as a variable internal standard. However, it is also pointed out that potassium can be used similarly, and this was favoured in this study. Rubidium and cesium are closely associated geochemically with potassium. Similarly, their behaviour in the arc follows that of potassium more closely than that of sodium, and potassium should be the superior internal standard. Some rubidium determinations obtained by a method using sodium as internal standard, have been shown to be subject to error. This is due to the fact that the Rb/Na ratio tends to change during the period of alkali distillation, so that erroneous concentrations will result if the exposure is terminated before all the alkali elements have distilled. (TAYLOR, ERLANK and GURNEY (1967).

An attempt to increase the sensitivity of the existing procedure by the use of a buffer was made. RUSANOV and KHITROV (1959) and IVANTISHIN et al., (1960) have reported success with this approach. It was found that the buffer, in general, tended to increase the background in the vicinity of the cesium line. Using a prism spectrograph, with glass optics, the background problem is already severe in this region, and adversely affects the precision and the detection limit. Buffering alone, there-

fore, did not seem likely to provide the added sensitivity which was sought.

The plane grating spectrograph is generally more sensitive than a prism instrument. Less of the energy of the signal is absorbed by the optical system within the instrument. The diffraction grating which operates by reflection is superior in this regard to a prism, which disperses incident light by transmission. Less light scattering occurs, and the intensity of background radiation in the infra-red region, is much lower than that in a prism instrument. Furthermore, a diffraction grating has a constant dispersion for all wavelengths, which is a function of the number of lines per unit length ruled on its surface.

However, despite these advantages, the only previous use of a grating spectrograph for the determination of the alkali metals, was that of ANNELL (1964), who reported a detection limit of 1ppm. for cesium in tektites. Greater use had not been made of the diffraction grating spectrograph for the determination of rubidium and cesium, probably because of the existence of second and third order interfering iron lines. However, these can be eliminated by using the Spex Industries red filter, Catalogue No. 9023R, which absorbs light of wavelengths shorter than 6000Å, and hence absorbs all second and third order spectra in the red and infra-red regions.

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A comprehensive investigation of the potential of a method utilising this filter was carried out. The recommended optimum arcing conditions which were derived, are summarised in Table 7. The method is a D.C. arc technique, carried out on a Jarrell-Ash 3.4m. plane grating spectrograph, and has been used to determine rubidium and cesium at concentrations lower than 0.1ppm. Lithium may be determined simultaneously.

Optical Alignment, Line Focus and Lens Arrangements

At the outset, the optical alignment of the spectrograph was checked and adjusted, to ensure that the instrument was operating at its maximum sensitivity. Optimum focussing of the instrument was obtained by visual inspection of the spectra obtained, over a range of focus settings.

Various lens systems designed to focus the emission from the arc onto the slit of the spectrograph, were studied with reference to the intensity and reproducibility of the spectral lines produced. Ideally, maximum intensity of the spectral line, with uniform illumination of the spectrograph slit, is desired. Although it was not possible to attain this, a suitable compromise can be made.

The length of the spectrograph slit (i.e. wedge setting) was enlarged to 20mm. compared to the normal 10½mm. Test spectra were taken for each of a variety of lens systems recommended by

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the Jarrell-Ash Company. The intensity of the most sensitive rubidium and cesium lines (7800Å, 7947Å and 8521Å) were measured both in the centre of the line, and at its top and bottom ends. It was quickly recognised that the maximum intensity was always obtained in the centre of the exposure. Furthermore spectral lines obtained from two-lens systems, whilst having satisfactory intensity, showed pronounced non-uniformity longitudinally. They also gave poor reproducibility due to arc wander; the image of the arc not always being focussed on the spectrograph slit.

Three-lens systems were clearly superior, and two of these are outlined in Figure 3. System (ii) is recommended for maximum sensitivity with a reasonable line shape, whilst (i) is recommended where sensitivity is not critical. The longitudinal intensity distribution of Rb 7947 for each of these two systems is represented in Figure 4.

Electrodes

Wide ranges of type and size of electrode were investigated in an attempt to improve sensitivity and precision. Electrodes with cavity sizes ranging from 2.3 x 2.5mm. to 4 x 8mm. were used. Increase in capacity generally improved the line intensity of the spectra. However, this was off-set by a loss in precision when using the larger electrodes due to the erratic behaviour of the arc. Scribner-Mullin carrier type electrodes were more stable,

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but excessive cyanogen interference arising from the burning away of the side walls caused interference on Cs 8521 and Li 8126.

Interference on Rb 7947 has been reported (AHRENS and TAYLOR 1961) but the other interferences have not been previously mentioned.

The use of carbon electrodes as compared to graphite was unsuitable for the same reasons of cyanogen band production. Centre post electrodes, undercutting, and varying wall thicknesses were all investigated. Ultimately it was found best to use a conventional type of electrode. Extra sensitivity could be obtained by exposing the photographic recording plate to two or more arcings of the sample (i.e. by superimposing).

The electrode selected as being most suitable had a 3.15 x 6mm. cavity size with a wall thickness of 0.80mm. It was packed to 1mm. from the top of the crater. The electrode was large enough to give a low detection limit and yet not so large as to introduce excessive arc wander and give poor precision. Superimposing gives added sensitivity when required and improves precision. The only limitation on the number of superimposings is the production of the interfering cyanogen bands with repeated arcings. A detection limit of less than 0.1ppm. has been obtained with three superimposings.

The detection limit is dependent upon the sample matrix,

in as much as a refractory matrix, containing little total alkali metal, tends to arc at a high temperature. This leads to the production of cyanogen bands in the emission spectrum. Cyanogen emission lines interfere with Cs 8521, Li 8126 and one of the rubidium lines, and affect the detection limit adversely.

In this study the lowest detection limits have been obtained with olivine melilitites, eclogites and basalts.

Carriers, Buffers and Fluxes

A wide variety of buffers, carriers, and fluxes were investigated, with particular attention to their effect on the sensitivity, precision, and accuracy of the alkali trace element determination.

Of these, two, CaF_2 and SrF_2, gave encouraging results. Addition of CaF_2 was studied in detail. The presence of this substance in the arc brought about several desirable effects.

The period of distillation of the alkalis was appreciably shortened. The intensities of all the alkali metal lines were enhanced.

The enhancement was greatest for lithium. This meant that the relatively insensitive line, Li 8126, became a usable analysis line at the concentration levels where the most sensitive line, Li 6707 is almost always self-absorbing.

Figure 5 shows the selective volatilisation curves of the

alkali metals in a pure sample. The time interval of the plotted points is 15 seconds. The alkali burn lasts for two minutes.

Figure 6 represents the selective volatilisation curves of the alkali metals in the sample used in Figure 5, arced under identical conditions, but mixed with 20% CaF_2. The time interval was not the same as in Figure 5 and this should be borne in mind when assessing the relative intensities of the lines in Figures 5 and 6. Nevertheless, it can clearly be seen that the distillation period is appreciably shortened, a factor of importance when superimposing several arcings on each other. Figure 6 also illustrates that the end of the alkali metal burn is followed by a short period in which calcium is the predominant cation distilling into the arc. This is distinguishable easily and is a convenient point at which to end the burn, preventing recording of excess cyanogen, the cyanogen emission being much greater after the end of the alkali phase.

Figure 7 shows the intensity enhancement effect of calcium fluoride on the alkali metal lines. The diagram shows the relative intensities of the lines obtained when 5, 10, 15, 20, 25 and 50% CaF_2 are added to the sample. These intensities are normalized with respect to those obtained from arcing the pure sample where the intensity of each line is taken as unity. From this figure the striking enhancement of lithium can be seen.

Potassium, rubidium and cesium are also enhanced by decreasing factors, in that order.

The approximate factors for the enhancement are 4 to 5 for lithium, 2 for potassium, and 1.5 for rubidium and cesium. The mechanism of these enhancement effects is not clear.

It was decided to add 20% calcium fluoride to each sample. In addition to producing the enhancement effects shown in Figure 7, this amount was also sufficient to act as a conventional buffer in removing matrix effects.

Cyanogen Interference

Cs 8521 and Li 8126 are liable to cyanogen interference. However, there are several lines in the cyanogen band spectrum close to and slightly greater in intensity than the interfering lines. These lines can be used to indicate whether or not a correction should be made for cyanogen interference.

An attempt was made to arc in an atmosphere free of nitrogen using a Stallwood jet. The only promising results were obtained with argon or argon/oxygen mixtures. However, as there is a line in the argon spectrum at 8521, this work was not pursued further. Helium or helium/oxygen atmospheres suppress the intensity of the alkali lines as well as reducing fractional distillation.

Standards

There is a lack of satisfactory natural rock standards at

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the concentration levels required; synthetic standards were utilised. The preparation of these is described in detail in the Appendix.

The use of the synthetic standards has been found to be satisfactory as long as an internal standard, such as potassium, is used. Straight intensity plots are systematically displaced due to the difference in density between the ultra-basic materials and the synthetic standards which leads to different weights of each being packed into the same size of electrodes.

This is illustrated in Figure 8 where the intensity of the Rb line 7947 is plotted against concentration for both natural and synthetic standards.

Precision and Accuracy

The method has been used to determine the lithium, rubidium and cesium contents of a variety of basic and ultrabasic materials. Precision, expressed as the relative standard deviation, is indicated in Table 8, using data derived by arcing one sample 12 times and by arcing a series of samples in duplicate. Also shown are precision data calculated without the use of potassium as internal standard, as it is possible to use the method without internal standardisation by simply plotting intensity against concentration if the potassium contents of the samples are not known. The differences in precision between columns (b) and (c)

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in Table 8 provide a direct indication of the efficiency of potassium as an internal standard. The precision obtained by the proposed method may be considered satisfactory and is attributed to the use of potassium as an internal standard, the technique of superimposing three arcings per exposure, the use of an electrode packing machine (Elpac) for filling the electrodes, and the addition of CaF_2 to the sample to promote a more effective distillation of the alkali metals into the arc.

To assess the accuracy of the method, the U.S. Geological Survey standard diabase rock W-1 (FLEISCHER & STEVENS 1962) was analysed as an unknown sample. Values of 22.8ppm. rubidium and 0.8ppm. cesium were obtained. The rubidium figure is close to the recommended value of 22ppm., while the cesium figure, although lower than the recommended value of 1.08ppm, was in better agreement with the most recent determinations available. (Table 9).

More recently, additional analytical data has been obtained as the method has been applied to the analysis of basic and ultrabasic silicates. Some of this is presented below.

Rubidium

Table 2 lists rubidium data in some mafic and ultramafic rock types. The optical spectrographic data were obtained using the experimental conditions described previously, including the use of three superimposings per exposure and the synthetic standards used in that study. The isotope dilution - mass spectro-

metric measurements were carried out on aliquots of the same powders. It is important to note that the isotope dilution measurements were obtained after the optical spectrographic measurements were calculated, each set of results being obtained independently of the other. The general agreement of the two sets of data with concentrations of as low as 0.2ppm. is noteworthy. The relative difference (i.e. percentage mean difference) of the optical spectrographic data from the isotope dilution data is approximately 5% and serves to demonstrate the accuracy of the method, as the isotope dilution technique is generally considered to be amongst the most accurate of those available.

These analyses were carried out by A.J. ERLANK and have been reported elsewhere, ERLANK, DANCHIN and FULLARD (1968). Inspection of the results contained in Tables 18 and 19 shows that agreement between the optical spectrographic and X-Ray fluorescence methods is likewise very good over the range 2-20ppm.

Cesium

Three new determinations of cesium in W-1 have been published since the figure of 0.8ppm. obtained by the spectrographic procedure was published. (GURNEY and ERLANK, 1966). They appear in papers by NICHOLLS et al., (1967), GORDON et al., (1968) and GAST (1968). These have been listed in Table 9, together with previously published determinations.

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Although the figure of 0.80ppm. is slightly lower than the average of 0.86ppm. calculated from the four newer determinations it lies well within the spread exhibited by the latter. Hence it may be concluded that the accuracy of the method for Cs is not seriously in question. It is also worth noting that the average of 0.86ppm. given above is some 20% lower than the previously recommended figure of 1.08ppm. (CABELL and SMALES (1957)).

Lithium

Comparison of results obtained by atomic absorption with those obtained by the optical spectrographic method suggest that lithium values of reasonable accuracy have also been obtained. Here, however, the position is less clear due to the fact that the synthetic standards prepared contain a lithium blank, which adversely affects both the useful detection limit and the accuracy of the determinations. The atomic absorption method utilised solutions of known Li concentration made up from a pure lithium salt. The spectrographic values were based on W-1 (12ppm.) and synthetic standards, in which the Li blank had been determined by atomic absorption. The results are presented in Table 10.

Discussion

Although there is only limited verification of the accuracy of the method for Li, and although evaluation of accuracy for Cs is based on one analysis, the Rb data presented suggest that the

accuracy of the method is adequate for most geochemical purposes. Coupled with the speed, precision and simplicity of analysis, the method has obvious advantages. Provision of accurately analysed natural rock standards would further improve the accuracy of the method as matrix affects would be reduced.

CHAPTER FIVE

SUMMARY OF ANALYTICAL PROCEDURES AND TECHNIQUES

The spectrographic procedure outlined in Chapter Four was used to obtain geochemical data on the ultrabasic material available. Although it appeared that sufficient sensitivity had been successfully achieved, the analytical data was checked by alternative methods, where these became available. Potassium and zinc quantitative results are also presented here, and the same cautious approach was used, although no optical spectrographic work was involved in their determination. A summary of the analytical methods used is best discussed element by element.

Potassium

The potassium content of ultrabasic xenoliths is low, whilst that of the host rock, kimberlite, is comparatively high. The range of concentration is approximately 0.01% to 3.5%. No natural rock standards of peridotite, eclogite or kimberlite with concentrations of potassium within this range, were known. No rock standards at all with potassium contents less than that of W-1 at 0.54% were available. The instruments available on which potassium determination could be carried out, were a flame photometer, an X-Ray fluorescence spectrograph and an atomic absorption spectrophotometer. The flame photometer lacked

sensitivity and is prone to inter-element effects in the excitation flame, which could become particularly significant at low concentrations in highly magnesian samples. (COOPER 1963, HAMILTON 1965). There were a number of X-Ray techniques, which could be investigated, but these were of unknown accuracy, whilst atomic absorption had not been used for the determination of potassium in ultramafic materials.

The accurate analysis of potassium was essential, both for its own importance, and as it was used as an internal standard in the optical spectrographic technique. Table 1 has shown that the older wet chemical analyses were not accurate, and no reliance was placed on these values (see AHRENS, PINSON and KEARNS, 1952).

A number of flame photometric analyses for peridotites, eclogites and kimberlites were kindly made available by G.W. BERG. Separate portions of some of these samples had been analysed by K. HEIER (1963), using both a refined flame photometric technique and gamma spectrometry. The agreement was good, but none of the samples had potassium contents less than 1000ppm. Additional analysed ultrabasic material was provided by DR. P. HAHN-WEINHEIMER, DR. A.M. STUEBER, and A.J. ERLANK, and these samples covered a range from 300ppm. to 1000ppm. Three ultramafic rock powders, low in potassium content, were sent to Dr. I. McDOUGALL of Canberra University, where they were analysed by the flame

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photometric procedure of COOPER et al. (1966). The standard peridotite, PCC-1, and the standard dunite, DTS-1 completed a suite of samples which was to provide the nucleus of the standards for the X-Ray fluorescence technique, which was ultimately adopted for analysis of all the ultrabasic samples.

No absolute method of potassium estimation was available. The successful determination of this element depended on obtaining good standards. It was resolved to investigate the possibility of using an atomic absorption method of analysis. Potassium analyses by the atomic absorption method have been reported to be quite accurate in silicates. Sodium ionisation interference occurs, but this can be eliminated by buffering when necessary. (BILLINGS and ADAMS, 1964; BILLINGS 1965).

However, no determination of potassium in ultrabasic materials had been reported, and the author's approach was, therefore, cautious.

Substances possess the property of being able to absorb characteristic radiations when dispersed as a vapour. These radiations are the same as those which the same substance can emit, and this is the basis of atomic absorption spectroscopy.

Compared to other spectrographic or spectrophotometric procedures, the general lack of spectral or chemical interferences is a primary advantage of the method. Although problems do

exist in the analytical method, they are usually amenable to solution. Excellent spectral resolution results in a high degree of element specificity and sensitivity is good.

One difficulty often encountered is curvature of the calibration graph with increasing concentration of the element determined. This was overcome by always diluting solutions sufficiently to ensure that the measured absorbance fell on the lower, straight, portion of the calibration curve. The cause of the curvature is often resonance broadening of the line, but it may also be brought about by having too broad an emission line, or by the increase in viscosity of the solution, with increasing concentration.

In the determinations reported here no evidence of either physical or chemical interferences was encountered. Such problems as arose were concerned with contamination of the solutions.

The determinations were carried out on a Perkin Elmer 303 Atomic Absorption spectrophotometer. The conditions were optimised and the instrument settings are listed in Table 11. (They were determined by M.J. ORREN, Department of Geochemistry, U.C.T).

A suitable weight of sample was decomposed by hydrofluoric and perchloric acid attack. The residue remaining on evaporation was taken up in 50ml. of 2N hydrochloric acid to yield a solution of 5-10ppm. potassium, if possible. The results were read off

a calibration curve obtained from accurately weighed out standard solutions of potassium salts.

Contamination was experienced from glass volumetric flasks. Ultimately the analyses were performed from solutions made up to the required volume by pipetting the appropriate amount of HCl directly into the platinum vessels in which the initial decomposition was carried out.

Potassium values in excellent agreement with the recommended figure for W-1 were obtained. The agreement between the values obtained for ultrabasic materials, and those obtained elsewhere using different methods for the same samples, was also good. (Table 12).

A total of forty six additional potassium determinations were then performed by this method, covering the complete range of rock types and potassium contents. These determinations were used to check the whole rock X.R.F. method discussed next. (Tables 13, 14 and 15).

An investigation of the possibility of using a simple X-Ray fluorescence technique for the potassium determinations yielded positive results. It was found that, provided the rock powder was extremely finely ground (-300 mesh), particle size effects were eliminated. Further, if the standards and the unknown samples were of the same rock type, mineralogical effects and

the difference in mass absorption coefficient were likely to be small. In practice, as can be seen in the results set out in Tables 13, 14 and 15, a potassium value obtained by this X-Ray fluorescence whole-rock technique varied from the average value by greater than 5% of the amount present in only one case. No more can be claimed for the other methods investigated. As the whole rock technique is the simplest, quickest and most sensitive available, it was used for all samples.

The sample powder was fine-ground to -300 mesh and briquetted under a load of 15 tons with a bakelite-boric acid backing, after the method of VOLBORTH (1963). The potassium line intensity and the background radiation were measured. Separate straight line working curves were obtained for eclogites, peridotites, kimberlites and melilitites. However, the curves for peridotite and eclogite were close to each other, whilst the standard diabase W-1 fitted on the melilitite curve. No mass absorption correction was applied, as the major element analyses were not yet available. However this should not influence the results greatly. Machine operating settings are set out in Table 16.

As an additional and final check, fifty samples were determined for potassium, using a technique similar to that of WELDAY et al., (1964).

The sample of -120 mesh powder preheated at 950°C to constant

weight (approximately 1 hour) was mixed with lithium tetraborate (1gm : 4gm), and fused in a Pt-Au $_{5\%}$ alloy crucible. The fusion temperature was 1050°C, and the sample and flux were maintained at this temperature for twenty minutes, swirling the melt every five minutes. The melt was poured into a red-hot, shallow, Pt-Au$_{5\%}$ dish heated over a bunsen. The dish was removed from the flame and allowed to cool on an asbestos pad placed on a hot-plate at 260°C. The resultant disc fell free from the dish after a few minutes and was annealed on the asbestos pad for thirty minutes. The samples were analysed for potassium by X-Ray fluorescence, with reference to W-1, treated similarly, as a standard. The instrumental settings will be found in Table 16. This particular fusion technique was chosen because of its good sensitivity and small blank which permitted the determination of potassium down to levels of less than 50ppm.

The object of the fusion is to remove mineralogical differences and to reduce differences in mass absorption coefficient. The method should be superior to the whole-rock technique, particularly when, as in this case, the major element composition of the samples is not known. However the agreement between all the methods was such that it is felt that the results presented in Section B of the Appendix are likely to be within 5% of the correct values. The preferred value is an average of those

obtained by the methods outlined here, together with the value of BERG where available. It should be noted that in the cases of the kimberlites and the melilitite basalts that it was necessary to apply a dead time correction to the counting signals of the X-Ray method, due to the generally high potassium concentrations in these rocks.

The fusion technique gave excellent results for most samples. However, certain kimberlites yielded low values. In every such case there was evidence that sample was lost by volatilisation in the preheating to 950°C. These apparent losses should be investigated further, and led to the results reported below:-

% K

SAMPLE	ATOMIC ABSORPTION	GAMMA SPECTROMETRY	FLAME PHOTOMETER	X.R.F. W.R.	X.R.F. FUSION
KDT 24	0.92	0.88	0.91	0.96	0.62
KDT 28	1.27	1.27	1.24	1.31	0.95
KDB 9	1.20	1.24	1.21	1.20	0.92
KBEL 2				1.59	1.36

In these four cases a brown film on the inside surface of the containing vessel, and a similar stain in the lip implied the creation of a dense gaseous phase in which some iron, and presumably potassium as well, had been carried out of the crucible, whilst it was being heated. Five determinations of potassium

in kimberlites, in which material loss did not appear to occur, yielded results in excellent agreement with other determinations:-

	% K	
SAMPLE NO.	PREFERRED VALUE	X.R.F. FUSION
KRV 14	3.26	3.26
KJAG 11	1.36	1.38
KK 2	0.43	0.42
XSF 7	2.28	2.28
KBEN 1	0.22	0.23

Rubidium

This element was determined in all the samples available.

The spectrographic procedure was used for rubidium determination in the ultrabasic xenoliths. These were checked wherever possible by the less sensitive, but accurate, X-Ray fluorescence method.

In the X.R.F. method the sample powder was fine-ground to -300 mesh, and briquetted under a load of 15 tons, with a bakelite and boric acid backing after the method of VOLBORTH (1963). The mass absorption coefficient (Table C 1) was estimated by the method of measuring the intensity of the Compton scattered portion of a MoKα primary beam (REYNOLDS 1963). Great care was taken in the measurement of the background radiation at the wavelength of the rubidium excitation to ensure that, even at low

levels of rubidium, the difference between the two could be accurately determined. Instrumental settings and further details are contained in Table 16.

In order to obtain sufficient accuracy, the samples with low concentration levels of rubidium were determined more than once. For instance, the values quoted for peridotites with less than 3ppm. are the average of ten individual values.

The detection limit of the X.R.F. technique was 0.7ppm. (WILLIS, 1968). Values less than 1.4ppm. were not considered in computing the preferred values quoted in the results. Apart from these cases the preferred values are averages of those obtained by the optical spectrographic and X-Ray methods. Agreement between the two methods is considered to be good. (Tables 18 and 19).

In the O.S. determinations if the potassium content of the sample was so low that the internal standard analysis lines were not visible, the rubidium determinations were calculated from the intensities of the rubidium lines alone. Comparison with samples where it was possible to determine rubidium by both methods indicates that these results are of satisfactory accuracy (Table 19, Columns (a) and (b)).

In the spectrographic procedure the two rubidium lines 7800 and 7947 are self-absorbing when more than 20ppm. of rubidium is

present in the sample. Samples with a high rubidium content were determined by X.R.F. analysis only.

Cesium

Cesium was determined by the optical spectrographic technique in all samples. No other suitable alternative method was available. The accuracy of the results is not well established, but was discussed in Chapter Four.

Cesium has been determined in all the eclogites, all the melilitites and a number of peridotites and kimberlites. The operating conditions were not identical for these various rock types, due to their widely differing alkali metal contents. The method outlined in Chapter Four was used for determination of the cesium, rubidium and lithium in the eclogites and peridotites. Kimberlites and olivine melilitites were arced under modified less sensitive conditions; these are summarised in Table 20.

The ultramafic peridotites behave very erratically in the arc, and those with low alkali content show no alkali metal phase of arcing. This is particularly so when the sample is serpentinised, as mentioned by AHRENS (1961). HOLYK (1952) suggests the addition of two parts of carbon to improve the burning quality of the discharge, but this is not applicable in the determinations under discussion, as a cyanogen line interferes with the cesium line at 8521Å. In the peridotitic samples, the appearance

of this cyanogen interference was, in any case, a problem. The results quoted are for samples in which the cyanogen band spectra did not appear. No attempt was made to correct for the interfering line in spectra where it did appear. In such samples, the cesium line intensity was usually so low that such corrections would have been impossible to make with accuracy.

The kimberlites were difficult to arc. This was due to the presence of serpentine and carbonates in the samples. Heating the loaded electrodes to 450°C and making use of a pre-ignition period in the arcing procedure improved the arcing characteristics. Nevertheless many samples extruded. Pre-heating to higher temperatures might improve these characteristics further, but care must be taken not to lose a volatile element such as cesium. At higher temperatures, too, the graphite electrodes tend to oxidise.

Lithium

Lithium was determined spectrographically by the same method as rubidium and cesium. Potassium is not as ideal an internal standard for lithium as it is for rubidium and cesium. Sodium would be better. Figure 6 illustrates that lithium takes longer to distil into the arc than potassium and that lithium line intensity will be reduced if the photographic exposure is not long enough. If the exposure is slightly too long cyanogen interference occurs. A high degree of operator skill is

required if accurate lithium analyses are to be obtained. The spectral line Li 8126 could not be used to determine lithium in many peridotites where the lithium concentration was less than 2ppm. In these samples cyanogen interference always occurred. The cyanogen line intensity was usually greater than that of the lithium line and no correction was attempted.

The results obtained were checked by a number of independent determinations by atomic absorption. (Tables 10 and 21). Agreement between the two methods was usually satisfactory.

Zinc

It may be recalled that zinc was one of the elements which an initial attempt was made to determine by the ion-exchange pre-enrichment optical spectrographic procedure of BROOKS (1960). A simple procedure making use of X.R.F. analysis has been used in this study. The method, which has successfully been applied to basic rocks (B.M. GUNN 1966) was similar to that used for rubidium, in the manner of mass absorption coefficient correction and sample preparation. The instrumental operating conditions are set out in Table 16.

There are two features which are worth noting.

Firstly the gold target tube contains Zn impurities, and a correction has to be made for this. This is done by measuring the intensity of the zinc peak obtained for a sample known to contain no zinc. This intensity will then be due to the impurity

in the target tube. The magnitude of the intensity is related to the intensity of a nearby characteristic line of the target tube. Measurement of the intensity of this characteristic line for each sample enables a prediction to be made of the amount of the observed zinc line intensity which is due to the target tube impurity.

Secondly, it was noticed that a newly prepared standard sample emitted a zinc line intensity some 6% lower than a sample of the same standard, which had been used many times. This was traced down to the presence of zinc in the oil used in the vacuum pump of the spectrograph. During the course of repeated use, the standard materials had become coated with a thin film of oil, and this was the cause of the added intensity. It was not possible to establish whether the zinc contaminant was an original constituent of the oil, or had been picked up from the atmosphere. The oil was replaced with a clean supply, zinc-free, and the appropriate correction made.

The zinc results were checked by atomic absorption spectrophotometry. BILLINGS (1967) reported that serious molecular absorption effects interfere with the determination of zinc in silicate rocks.

No such effects were observed in the analysis of amphibolites (BURRELL 1965). In view of the excellent agreement between the

two techniques used in this study, it would seem that any absorption effects were minimal. (See Table 22).

Contamination of zinc solutions in plastic bottles was thought, after investigation, to be due to the use of zinc stearate as a lubricant in plastic moulds during manufacture of the containers. The contamination continued over an extended period and no amount of leaching seemed to be able to clean the bottles completely.

Atomic absorption makes use of extremely dilute solutions and storage of the dissolved samples prior to analysis was a major problem. It was overcome here by removing the storage step. Analyses were carried out on solutions made up to volume by pipette in the same platinum crucibles in which the $HF/HClO_4$ attack was performed. After stirring and allowing to stand, this solution was analysed directly. A standard reference sample was always determined simultaneously.

The only draw-back of this method was that it limited the number of determinations at one time for each element to the number of platinum vessels available.

An attempt to determine zinc by an addition plot method using X.R.F. analysis was unsuccessful. Although good straight line working ourves were obtained, the agreement with the other two methods was not satisfactory. (Table 22).

Thorium and Uranium

The kimberlite samples were analysed for potassium thorium and uranium by means of gamma ray spectrometric measurements of natural radio-activity. The sample preparation was limited to crushing and weighing. No chemistry was involved. The technique used was similar to that of ADAMS et al. (1958).

The analyses and the computing of the results were carried out by Dr. R.D. CHERRY and J.B.M. HOBBS of the Physics Department, Cape Town University. The potassium analyses were not considered to be as accurate as other methods reported here, but a comparison of the values obtained by this and other methods shows a generally fair agreement. (See Table 17). The thorium and uranium analyses are considered to be more accurate.

This method could not be used to determine the same elements in the peridotites or eclogites as the levels of concentration of the elements was generally too low to permit accurate analysis. The more sensitive α counting technique (CHERRY 1963) was tried as an alternative but two difficulties have not been overcome.

Firstly standardisation of the method has proved difficult because of the absence of reliable thorium and uranium standards at low levels of concentration. Secondly, it has not been found possible to grind silicate material finely enough to remove all particle size effects. If these two problems can be resolved

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satisfactorily, there is no reason why the technique should not be applied successfully.

CHAPTER SIX

DISCUSSION OF THE NEW DATA

The discussion of the new data is presented in a series of sections and sub-sections.

Section one deals with the potassium rubidium and cesium determinations. Brief discussions of the relevant geochemistry of the three elements are followed by sub-sections dealing with the results obtained for the various rock types.

Section two is confined to a discussion of the zinc determinations and is sub-divided along similar lines to Section one.

Section three deals with the lithium results obtained and Section four with the thorium and uranium analyses of kimberlites.

SECTION ONE

(a) Potassium

Potassium, a strongly lithophile element, is enriched in the crust relative to the whole earth or the mantle. It is mainly contained in feldspar, mica and feldspathoidal minerals. Its concentration increases with fractionation and this increase is relatively greater than that of sodium, resulting in an increasing K/Na ratio. The potassium content of ultrabasic material, thought to be derived from the mantle, is usually low.

HAMILTON and MOUNTJOY (1965) have reported that the average content of 42 alpine ultramafic rocks was 340ppm. K_2O. MORGAN

and GOODE report much lower values for ultrabasic material as does MAKSIMOVIC (1967) for Yugoslavian ultramafic rocks. MURTHY and STUEBER (1967) report generally low potassium contents for a variety of ultrabasic rocks with the kimberlitic inclusions having rather larger amounts than the other rocks studied.

(b) Rubidium and the K/Rb Ratio

Rubidium does not form any mineral of its own, but is almost always found to substitute for potassium in potassium minerals. It is seldom considered on its own geochemically and far more interest is attached to the K/Rb ratio than to the absolute abundance of rubidium itself. Potassium and rubidium provide such a good example of geochemical coherence because of the similarities in ionic charge, ionic size and bonding properties of these elements. AHRENS et al., (1952) were the first to demonstrate the general coherency existing between potassium and rubidium. TAYLOR et al., (1956) revised the earlier data, and the geochemistry of the alkali metals including potassium and rubidium was reviewed by HEIER (1964).

Several important conclusions were reached in these papers and the geochemical significance of the two elements was emphasised. Firstly it was suggested that the K/Rb ratio in common igneous rocks does not vary greatly. Most values fall in the range 115 - 460, and a crustal average of 230 was proposed and

is generally accepted. In addition the K/Rb ratio in basic rocks was thought to be slightly higher than in acid rocks, whilst the ratio showed a marked decrease only in the final stages of differentiation, or in highly fractionated granitic rocks.

Finally, the K/Rb ratio of basic rocks was similar to that observed for the chondrites, ($\sim$300), and this was taken to support the chondritic earth model concept.

However, more recent data indicates that the K/Rb relationship in basic rocks is not so simple as was previously thought. High K/Rb ratios of greater than 500 have been reported in lavas from oceanic islands, (LESSING et al., 1963; McDOUGALL and COMPSTON 1965), whilst still higher ratios, up to 2000, and similar to those of achondritic meteorites, have been reported for oceanic tholeitic basalts (GAST 1965, TATSUMOTO et al., 1965).

It has been shown that these high values are also present in some continental areas (ERLANK and HOFMEYR 1966 and 1968; ERLANK et al., 1968; PAYNE and SHAW 1968).

The importance of mineralogical control of the K/Rb ratio has been demonstrated (HEIER 1965; TAUSON 1965; HART and ALDRICH 1967; ERLANK 1967; ERLANK et al., 1968). It has been suggested that this aspect may be of particular importance in rocks where there are no primary potassium minerals present. In such cases K^+ and Rb^+ may have to substitute in Na^+ sites

in the minerals present and Rb^+ on account of its larger size may be preferentially excluded. STUEBER and MURTHY 1966, have produced accurate potassium, rubidium and K/Rb data for a variety of ultramafic materials. The K/Rb ratio in potentially mantle derived rocks has been reviewed (MURTHY and STUEBER 1967).

However, at the present time no clear picture has emerged.

The observed variations of the K/Rb ratio in basalts have not been explained in terms of processes of magma generation which are known to have their origins in the upper mantle. Nor can any precise value be assigned to the K/Rb ratio of the upper mantle.

In the light of these recent developments, the analyses reported here assumed greater importance. Whilst the conclusions drawn from them do not solve the problems mentioned, they do, if correct, bring attention to certain possible pitfalls which have not been generally recognised hitherto.

(c) Cesium and the K/Cs Ratio

The geochemistry of cesium is not as well known as that of the other alkali metals, evidently due to its low abundance, particularly in basic and ultrabasic rocks.

Cs^+ substitutes for K^+ in potassium minerals, in a manner analagous to Rb^+, but because the difference between the radii of K^+ and Cs^+ is greater than between K^+ and Rb^+ the K/Cs ratio

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varies more widely than the K/Rb ratio.

As a result of this the K/Cs ratio may be of use in accentuating trends shown by the K/Rb ratio. This is borne out in some of the results presented here. The absolute cesium values and K/Cs ratios determined in ultrabasic samples have proved more useful in solving certain of the geochemical problems posed, than any of the other data obtained.

(d) Potassium, rubidium and cesium in South African Kimberlites

Kimberlite is an undersaturated rock. It contains unusually high amounts of potassium for a rock of such basicity and has a high K/Na ratio. DAWSON (1960) has summarised the available major element data on kimberlites, (Table 23). In this work the overall average content of potassium in kimberlites is 1.4% K, (1.68% K_2O), which is between DAWSON's value for micaceous and basaltic kimberlites. Previous data on South African kimberlites for rubidium and cesium appears to be lacking.

Several points are worthy of comment.

The potassium content of kimberlites varies widely, and whilst particular pipes or dykes may have a characteristically high, low or intermediate potassium content, individual samples of material from the same occurrence, may vary considerably. Rubidium, unexpectedly, varies in concentration to a much smaller extent, whilst cesium is extremely erratic, both within one

occurrence and from one occurrence to another. Certain kimberlites, those of Roberts Victor, Du Toit's Pan and Jagersfontein in this study, seem to contain considerably lesser amounts of cesium than others (e.g. Bultfontein, Wesselton and De Beers). The cesium contents of four kimberlites from the De Beers Mine are quite abnormally high.

It is interesting to note that the De Beers Mine is also known to contain abundant zeolites. The irregular distribution of cesium may be due to its concentration in these minerals which are known to have ion exchange and molecular sieve properties. They would tend to adsorb a large mobile ion such as Cs^+ preferentially.

The K/Rb ratios of the kimberlites are mostly within the range from 115 to 460, common for continental igneous rocks (ERLANK 1967). However, the observed ratio is usually less than the generally accepted crustal average of 230. This ratio shows no tendency to decrease with increasing potassium content. Any trend, if there is one, is in the reverse direction. (See Figure 9).

The K/Cs ratio varies from pipe to pipe but is generally lower than that of the average basic rock (HEIER 1964; AHRENS and EDGE 1961). DAWSON (1967) gives average values of 12ppm. cesium and 11ppm. rubidium in Russian kimberlites. Whilst the

average value of these two elements present in South African kimberlites is higher, cesium does appear to be enriched relative to rubidium and in particular samples the amount of cesium present approaches the amount of rubidium.

(e) Potassium, rubidium and cesium in peridotite nodules from kimberlites

A study of the data on peridotites leads to the important conclusion that most of the potassium, rubidium and cesium in peridotites is derived from the kimberlite matrix. Abundance data obtained from a whole rock study such as this, should not therefore be used, directly, to estimate the abundance of these elements in the upper mantle. The K/Rb and K/Cs ratios are similarly affected.

The xenoliths studied were not found in situ, and so it is only possible to relate their geochemistry to that of the host kimberlite in a general way. In any large occurrence, there is evidence of repeated injections of magma giving rise to kimberlite having different mineralogical and chemical characteristics to the adjacent material, and this leads to the variations reported in sub-section (d). In addition whilst the suite of over one hundred peridotites examined is a comprehensive one when taken as a whole, it has certain defects when broken down into groups of xenoliths from particular occurrences.

Only two samples are from Roberts Victor Mine, whilst only two samples from Jagersfontein are garnetiferous. It is unlikely that the samples from any one locality are representative either statistically or mineralogically. WAGNER (1914) has listed twenty nine different mineralogical combinations found in ultrabasic xenoliths. Many of these combinations can show widely differing proportions of some of the minerals, and to represent all types and variations is impossible.

Notwithstanding these shortcomings, it does appear to be worth while to consider both the peridotites and the kimberlites in groups from each locality, as well as to consider them as a whole.

The average potassium content of the xenoliths is 0.14% K. The distribution is illustrated in the form of a histogram in Figure 10. There is no apparent difference between the potassium content of the harzburgites and the garnet lherzolites (Figure 11). There is no relationship between the amount of potassium in the peridotites and the amount in the host kimberlite (Table 24).

In view of the stated conclusion that the bulk of the potassium is derived from the kimberlite, this may be surprising. However, the actual amount of potassium in a nodule depends largely on the amount of phlogopite, which is present. This

has been shown to be true by BERG (1968), using a semi-quantitative X-Ray diffraction technique for estimating the amount of phlogopite present (Figure 12). It is interesting to note that extrapolation of the potassium-phlogopite relationship indicates that the amount of potassium in a mica-free peridotite would be of the order of 150-200ppm.

Secondary phlogopite has been reported in peridotite nodules by WAGNER (1914), WILLIAMS (1932) HOLMES (1937) and DAWSON (1962) amongst others. HOLMES, in particular, dealt with the aspect of phlogopitisation at length and was left in no doubt that the phlogopite originated by metasomatic replacement of the peridotite minerals. A comparative study of a series of specimens from a harzburgite to a phlogopite rock, including passage types, showed that throughout the series the phlogopite exhibited the same range of peculiarities.

" In the early stages, it is connected with the veins that surround and traverse other minerals; the veins never cut the phlogopite, though they are themselves locally, and finally entirely phlogopitised. Enstatite and bronzite become almost wholly replaced before olivine is appreciably attacked, and olivine appears to be less resistant than chrome diopside and the earlier alteration products of garnet."

A phlogopite rock, possibly representing an extremely

phlogopitised nodule, was analysed by HOLMES and contained 8.52% K_2O. Three analysed by the author contained 8.0%, 7.2% and 6.8% potassium respectively. The average potassium content of a collection of peridotites will depend on the number of phlogopitised speciments included, and their degree of alteration, rather than on the kimberlite from which they came. The average potassium content of the peridotites (Table 24) must therefore be considered in this light.

Greater significance is attached to the fact that the K/Rb ratio and the amount of cesium present in a suite of nodules appear to reflect the characteristics of the host kimberlite. This can be seen by comparing the data obtained for kimberlites and peridotites and presented in the Appendix (Section B.). Table 24 summarises the position and Figure 13 is a graphical representation of the apparent approximate co-variance of the host and xenolith K/Rb ratios.

The relationship between the host kimberlite and the nodules is well illustrated by the cesium analyses for these materials. At Wesselton and De Beers Mine, where the kimberlites have a consistently high cesium content, the cesium content of some of the peridotites is also high. The amount of cesium present in some of these peridotites is so high and the resultant K/Cs ratio so low, that it is inconceivable to regard the cesium concentra-

tion as primary. It is probable that this element is accommodated in the phlogopite structure, but this is not necessarily so. As has been shown in the case of the eclogites from Roberts Victor Mine, (discussed in the next Section), the secondary alteration of high pressure minerals such as pyroxene can alter the geochemical distribution of cesium considerably.

(f) Potassium rubidium and cesium in eclogite nodules from kimberlites

The suite of eclogites studied consisted of twenty three samples, sixteen of which were from the Roberts Victor Mine. One further specimen is almost certainly from the same locality. The analytical results indicate that these rocks should be dealt with separately from the six from elsewhere.

(i) The Roberts Victor Mine Eclogites

Remarkably high concentrations of cesium relative to potassium were found in these eclogites. Rubidium, however, did not show an enrichment relative to the crustal average of 230. The K/Cs ratio ranged from 150 to 7288 giving an average value of 1810, whilst the K/Rb ratio varied from 222 to 552 and the average value was 367. Six fresh specimens of Roberts Victor kimberlites have K/Rb ratios within a narrow range of 230 to 265 and average at 242. The average K/Cs ratio for this material was 5000 and the average cesium concentration was 6ppm.

Whilst a correlation between potassium and rubidium was found, no correlation is apparent between potassium and cesium. The average cesium concentration of 5.9ppm. is extremely close to that obtained for the kimberlites. These three features are illustrated in Figures 14 and 15.

Further investigations by BERG (1968) have shown that the principal host for cesium in these rocks was a turbid alteration product of pyroxene, which he identified as having an analcime type structure. His data also indicate that the same zeolite has a high K/Rb ratio.

He proposed that this secondary zeolite was formed at a late stage under near surface conditions, and links its formation to the period of deep surface weathering of the kimberlite which produces yellow ground.

BERG (1968) also agrees with HEIER (1963) that secondary phlogopite contaminates these eclogites with potassium, rubidium and cesium. After careful consideration of the thin section evidence BERG writes:-

"If the eclogite nodules are considered to be xenoliths from the mantle or lower crust, and one common generation of brown mica is assumed, the present evidence leads the writer to suggest that the mica probably formed by metasomatic alteration of the eclogite while their host kimberlites were in a

fluid state under appropriate conditions of temperature and pressure.

In this case the secondary phlogopite may be expected to have a K/Rb/Cs relationship analagous to that of the fresh host kimberlite."

Finally, BERG has shown that the primary unaltered pyroxene of these eclogites can have a potassium content as high as 0.12% and a K/Rb ratio greater than 2000. The data of BERG (1968) on pyroxenes and zeolites is reproduced in Tables 25 and 26.

An attempt to show that the alkali metal content of these eclogites was labile by means of a leaching experiment indicated that appreciable quantities of these elements were not removed unless acid solutions were used as leaching agents. As it was expected that such acid leaching would destroy the analcime type secondary alteration, and possibly attack the phlogopite as well, no special significance was attached to this result.

A manual scan on the electron microprobe has confirmed that potassium in RV 377 occurs along the grain boundaries, in mica, in the pyroxene alteration product and to a lesser extent in the garnet alteration product. (Figure 17). It was also observed that potassium was not present to any appreciable extent in unaltered garnet, but occurred in material in cracks in that mineral. The distribution of potassium along grain boundaries

is analagous to that of uranium and thorium in an eclogite inclusion from the Delegate pipe, Australia. (KLEEMAN and LOVERING 1967).

The original K/Rb/Cs relationship in these eclogites has apparently been drastically altered by two secondary processes. The first of these, introduction of secondary phlogopite, would introduce potassium, rubidium and cesium, in proportions which would probably be lower than the primary K/Rb and K/Cs ratios of this rock, which may be indicated by the omphacite with a K/Rb ratio of $>$ 2000. The second alteration process would further increase the cesium content and lower the K/Cs ratio. The rubidium content was probably lowered and the K/Rb ratio certainly raised whilst the effect on potassium is not certain. The resultant relationship has no resemblance to the original except by chance.

The Roberts Victor Mine is readily accessible and has been worked intermittently since 1906. Eclogite is exceptionally abundant and has been distributed to Laboratories all over the world, and many analyses have been reported. Data pertaining to whole rock eclogite studies from this occurrence must be treated with scepticism.

(ii) Crown Mine Eclogite

One small sample, with the pyroxene badly altered, seems to

be analagous to the Roberts Victor material.

(iii) Jagersfontein Eclogite

Two samples of eclogite from this locality are much fresher than the Roberts Victor material, but nevertheless show some degree of phlogopitisation.

(iv) Tanzanian Eclogite

Three specimens, two from Dodoma Mine, and the other from Wilsons Prospect, Shinyanga, have been examined. The two specimens TAN 501 and TAN 503, which are the freshest kimberlite eclogite material available, have K/Rb ratios of 250 and 239 respectively and K/Cs ratios of 7,500 and 7,860.

There are no obvious secondary alteration products in either of these two rocks. TAN 1 and TAN 501 are remarkable for the presence of diopside exsolved in garnet, possibly due to a sudden release of temperature and pressure, and may be derived from great depths in the mantle (RINGWOOD, 1967). TAN 503 is a kyanite eclogite.

(g) Potassium, rubidium and cesium in olivine melilitites

Potassium, rubidium and cesium have been determined in some South African olivine melilitites. The K/Cs ratios which are reported for several of the thirteen samples are extremely high. HEIER (1964) has inferred ratios up to 94,000 in rocks from the Skaergaard complex, and TAYLOR and WHITE (1966) have reported a value of 22,700 for a Japanese andesite. SMALES (1964) has reported high K/Cs ratios in meteorites. However values as high as those reported here for the Spiegel River melilitite are exceptional, as is the strong positive correlation between the K/Rb and K/Cs ratio for the melilitites of the Sutherland occurrence (Figure 16).

The olivine melilitite from Spiegel River was collected from a pipe which cuts conglomerates and sandstones of the Cretaceous Uitenhage series. It has been described by ROGERS and SCHWARZ (1900) and HOLMES (1936) amongst others. Olivine occurs as idiomorphic crystals, many of which are corroded and embayed by the groundmass. Serpentinisation is very slight and the whole rock remarkably fresh. Perovskite and ore minerals are present and melilitite ranges in size from micro-porphyritic tabular crystals to minute lathes and interstitial patches in the ground mass. Flow structure can clearly be seen. The ground mass is rich in augite; nepheline, leucite, apatite and zeolites also

occur. High K/Cs ratios were obtained for each of the three samples of this material in which it was estimated.

The melilitite from Ashton, which occurs as a plug cutting shale of the Bokkeveld Series in the Cape System rocks, is similar to the Spiegel River material, but not quite as fresh. Again high K/Cs ratios were obtained.

The samples from Sutherland Commonage are of a more complex origin. There have been at least two phases of intrusion forming a sheet, and an associated dyke, and a secondary stage of hydrothermal alteration (GERRARD 1958). However, high K/Cs ratios were obtained for material collected from the area of the sheet. Three samples of the dyke, SU. 1, 2 and 3, have much lower K/Rb and K/Cs ratios. Secondary mica is much in evidence in thin sections of these three rocks.

It would appear as though a high K/Cs ratio is a characteristic of olivine melilitite.

Leucite is one likely host mineral for much of the rather high potassium content of the rock. If this is so, the small amount of cesium present is not due to an absence of suitable cesium sites in the minerals present.

FORNASERI and PENTA (1960) have shown primary leucite to contain 169ppm. Cs and 1791ppm. Rb. The structure of leucite is based on a framework similar to that of analcite and pollucite.

Potassium occupies a position in leucite equivalent to that of cesium in pollucite (DEER HOWIE and ZUSSMAN 1966).

HEIER (1964) has reported that potassium-feldspars with a high temperature of formation have low concentrations of cesium. It may be that the K/Cs ratios observed in melilitites are caused by a similar temperature effect, for olivine melilitites are thought to be high temperature intrusions.

Contrary evidence is provided by EUGSTER (1955) in experimental work on the cesium-potassium equilibrium in the system sanidine-water, in which he shows that cesium enters into the sanidine structure more easily at high than at low temperatures. At temperatures higher than 700°C the cesium content of the sanidine is greater than that of the fluid phase in equilibrium with it.

It may be concluded that at this stage it is not possible to infer either the K/Cs ratio of the source magmas or the source regions of olivine melilitite.

The strong correlation between the K/Rb and K/Cs ratios of the samples from the Sutherland occurrence is unusual. Cs^+ is a much larger ion than K^+ ; Rb^+ , whilst larger than K^+ is within the 15% size difference, which, as a general rule, will allow two cations of equal charge to substitute freely for one another in silicate structures. Consistent with this, cesium has a very

large dispersion in granites compared to rubidium and potassium (AHRENS 1954(a), 1954(b)). In the case of the Sutherland olivine melilitite, cesium, rubidium and potassium are varying independently, but the variations in K/Rb and K/Cs ratios are related linearly. A reworking of the original alkali distribution by secondary hydrothermal processes in the dyke material could be an explanation. The hydrothermal alteration product, mica, would have to retain cesium and rubidium relative to potassium, whilst it appears that some alkali metal content may have been lost. The dyke rocks have the lowest potassium concentrations, whilst the rubidium and cesium contents are not very different from those of samples of material from the sheet. This is consistent with the proposed explanation.

(h) Potassium, rubidium and cesium in miscellaneous samples

The eclogites from non-kimberlite environments show a greater range in their K/Rb ratios than other rocks analysed in this study. The values range from 217 to 860. These figures are in fair agreement with HEIER (1966) who reports a variation from 138 to 667 in six similar samples. The potassium content of these rocks is lower than that of the kimberlitic eclogites, and is particularly low in the fresh Norwegian material.

(j) Potassium, rubidium, and cesium in American Kimberlites

The values reported show that the six small samples analysed have a high potassium content and high K/Rb and K/Cs ratio when compared with the South African kimberlites.

Zinc

The geochemical distribution of zinc is controlled by several properties of the element, in particular the covalent nature of the Zn-O bond, its preferred tendency for tetrahedral co-ordination and the similarity in size of the Zn^{2+} ion to Mg^{2+} and Fe^{2+}.

Zinc from gabbroid magmas is often concentrated in oxidic ore minerals such as ilmenite, magnetite and chromite. Values of 3000ppm., 1000ppm. and 10,000ppm. respectively are reported by GOLDSCHMIDT (1954). Local concentrations of zinc in various other minerals are known but these are of little geochemical significance, compared with the association of zinc with magnesium and iron rich micas, amphiboles and pyroxenes, which are the main carriers of zinc in normal igneous rocks.

Biotite mica usually shows the highest content of zinc among the common rock forming minerals. Phlogopite mica also accepts zinc, although less readily. The preferential concentration of zinc in biotites and hornblendes has been sought in the nature of the structure of these minerals. WEDEPOHL (1953) noted the presence of (OH) groups in these minerals. He suggested that the presence of ions such as OH^- and F^- made it possible for zinc to assume octahedral co-ordination, and zinc is incorporated more easily into the silicate lattice.

NEUMANN (1949) had noted that zinc commonly occurred in four fold co-ordination although geometrical considerations predict octohedral co-ordination. Under unusual conditions such as those obtaining at Franklin Furnace, New Jersey, zinc does assume octohedral co-ordination in rare minerals such as larsenite ($Pb\ Zn\ SiO_4$).

TAUSON and KRAVCHENKO (1956) found that zinc was present in biotite in a form in which 80% of the element was extractable with HCl. They concluded that this zinc was present as minute sphalerite inclusions in the mica.

However, numerous results confirm that zinc, in rocks of magmatic origin, follows the ferromagnesian silicates. An interesting example of this is presented in Table 29 where whole rock and some mineral analyses are presented for four noritoids. Zinc is most highly concentrated in the magnetic fraction, and also in the hypersthene and the biotite. Surprisingly the concentration of zinc is higher in hypersthene than in biotite. The co-existing plagioclase feldspars contain very little zinc. (Also Tables 27, 28 and 30).

Zinc determinations in a suite of rocks from the Bushveld Igneous Complex illustrate further the role a mafic mineral may play in the distribution of zinc. Table 31 illustrates the effect of a decreasing amount of mafic minerals.

The average value for the zinc content of basalts of 105ppm. (TUREKIAN and WEDEPOHL 1961) has been well substantiated. RADER et al (1963) have analysed 124 American basalts and obtained an average of 99ppm. GUNN (1966) obtained an identical average for 30 Antarctican basalts. The author has determined zinc in 200 basic rocks from continental and oceanic environments and ranging in variety from dolerite and gabbro to trachyandesite, and obtained an average of 107ppm.

There are few analyses of ultrabasic materials by comparison. The figure of 50ppm. quoted by TUREKIAN and WEDEPOHL is the average of six determinations by WEDEPOHL (1953), of zinc in olivine rich rocks, none of which were from a kimberlitic environment. BROOKS (1960) reports 64ppm. and 29ppm. zinc from an eclogite and a peridotite respectively. Accurate zinc analyses in kimberlite do not appear to have been published previous to this study.

(a) The Kimberlites

The zinc content of the kimberlites is remarkably constant considering the nature of the rock, the varying freshness of the samples, and the variation in concentration of many of the major and volatile elements. This constancy is accentuated when the samples are considered as groups from each locality. (Figure 21). This is a feature of basalts also, and is well illustrated by

RADER et al (1963).

(b) The Peridotites

The 101 peridotites analysed in this study show an astonishingly small spread of zinc values. Ninety one contain between 30 and 40ppm. zinc, eight between 41 and 47ppm. and two have 22 and 23 ppm. respectively. (Figure 22).

The eight nodules with zinc concentrations of 41-47ppm. have a considerable amount of secondary phlogopite in them. Seven have potassium contents greater than 0.2% and it is felt likely that a small amount of zinc has been concentrated in this secondary mica and that it was derived from the kimberlite which contains more zinc than the xenoliths.

The two samples which contain the lowest zinc have been extensively serpentinised without the formation of phlogopite. It is suggested that zinc has been leached out of these two samples, in a manner analagous to that observable in the determinations of zinc in Roberts Victor yellow-ground.

Despite the much higher magnesium and iron content of peridotites when compared with basaltic rocks the zinc content of these samples is low. This is indicative of the fact that the geochemical distribution of this trace element is governed by properties other than similarity of size and charge.

The average zinc content of the peridotites is 34.6ppm. If they are a fair representation of upper mantle material, then, even allowing for the fact that the average may be a little high due to the inclusion of some altered samples, the zinc content of the upper mantle must be close to the value of 34ppm.

It has been suggested that the harzburgite xenoliths may represent the most depleted mantle material and that the garnet lherzolites represent the least depleted mantle material brought up in the kimberlite pipes (e.g. GREEN 1968). If this is so and garnet lherzolite is to produce basalt (Zn ~105ppm) by partial melting, leaving behind a harzburgite assemblage, then the average garnet lherzolite should contain more zinc than the average harzburgite. As there is little difference in the potassium contents (and hence the degree of phlogopitisation) in the representatives of these two groups (See Figure 11) it might be expected that any original difference in zinc content will still be apparent, when considering each rock type as a group.

Inspection of the analyses obtained shows considerable overlap between the two groups.

The average value of the zinc content of garnet lherzolite (37 determinations) is 36.5ppm. and that of harzburgite (28 determinations) is 33.0ppm. The difference although small is approximately 10% of the higher value. In view of the uncertainty

introduced by the possible effect of secondary alteration, it is not proposed to draw any conclusions from these averages except to observe that they are not inconsistent with the suggestion made above.

(c) The Eclogites

The zinc results reported in eclogites have a much greater scatter than those for other rock types, ranging from 12ppm. to 77ppm. (Figure 22). A large range in major and minor element composition seems to be a feature of these rocks. The average zinc content of 53ppm. is close to the average for kimberlites and significantly less than the average for basalts, of 105ppm. CZAMANASHE and DESBOROUGH (1967) have reported the presence of primary sulphide aggregates of Fe-Ni-Cu in eclogite from the Roberts Victor Mine. The aggregates occur between the grains, and also in the grains of the primary garnet and omphacite. No correlation between the major mineral phase composition of the nodules and the zinc content has been found, and the presence of sulphide accessory minerals may be significant.

(d) Olivine Melilitites

The zinc contents of the olivine melilitites are very similar to those of basaltic rocks in general.

(e) Miscellaneous Samples

The eclogites from non-kimberlitic environments have a large scatter of zinc content, ranging from 5ppm. to 186ppm.

(f) American Kimberlites

The six American kimberlite specimens have a distinctly higher zinc content than their South African counterparts.

SECTION THREE

Lithium

Li^+ is much smaller than the other alkali metal ions, and is unable to occupy similar positions in crystal lattices. The ion enters six fold co-ordination positions and is thought to substitute for Mg^{2+}. However, there is no close association between the two elements and the Li/Mg ratio increases markedly with fractionation. This was first observed by STROCK (1936), and has been confirmed by other workers. WAGER and MITCHELL (1951) show that in the Skaergaard Igneous Complex the lithium concentration in late crystallising olivines and pyroxenes from the ferrogabbro rises to 15ppm. and 50ppm. respectively. NOCKOLDS and ALLEN (1953, 1954, 1956) have shown that an increase in the Li/Mg ratio was a common feature in all the magmatic series studied, although the absolute amount of lithium may decrease in the most differentiated rocks, reflecting the decreasing concentration of the magnesium host minerals. HEIER (1964), assessing the available data, shows that the minerals orthopyroxene-clinopyroxene-amphibole-mica form a series in which there is a regular increase of lithium concentration and of the Li/Mg ratio. Two reasons are advanced for this.

(i) The structure of the mica lattice is most favourable for the Li^+ ion, and

90/...

(ii) during replacement of Mg^{2+} by Li^{+} the structure suffers a loss in energy. Therefore no considerable replacement can take place until a relatively low temperature has been reached. This is also the stage when mica is the important femic mineral.

The lithium content of ultrabasic rock is low, (STROCK 1936, PINSON AHRENS and FRANCK 1953). TUREKIAN and WEDEPOHL give a value of less than 1ppm. MAKSIMOVIC (1967) reported an average of 0.5ppm. lithium in 57 fresh ultramafic rocks; pyroxenites, lherzolites, harzburgites and dunites. The range of concentration was from less than 0.3ppm.-4ppm. The lithium content varied with the mineralogy of the sample, and increased from dunite to the more acid types. Lithium was found to be enriched in some serpentinised ultramafic rocks; 19ppm. lithium was reported from a serpentinised harzburgite.

On the basis of all the available data, MAKSIMOVIC recommended an average lithium content for four ultramafic rock types.

		Li (ppm)
(1)	Dunite	0.3
(2)	Harzburgite	0.3
(3)	Lherzolite	0.7
(4)	Enstatite pyroxenite	1.5

The lithium determinations reported in this thesis are not as precise or comprehensive as those reported for potassium,

rubidium and cesium. This is due to the experimental difficulties discussed in detail in Chapter Five. The detection limit for lithium in peridotites was 2ppm.

In a total of one hundred and one peridotites, eighty-three have a lithium content of less than 2ppm. Eighteen contain 2ppm. of lithium or more. These eighteen samples often show relatively abundant phlogopite and have high alkali metal and zinc concentrations. There seems no doubt that the lithium in these samples is largely of secondary origin.

Lithium is irregularly distributed in the kimberlites, and the amount is high for an ultrabasic rock. The Roberts Victor kimberlite seems to have a particularly high lithium content.

These high lithium values are reflected in the eclogites from Roberts Victor, which also have greater lithium contents than the eclogites from other occurrences. The eclogites in general have higher lithium contents than the peridotites, and appear to lie in an intermediate position between the peridotites and the associated kimberlite.

It is not possible to indicate whether the determinations reported are a primary or secondary feature of these eclogites. BERG (1968) has shown that the sodium content of the eclogites is largely primary, and it is possible that this is true for lithium also. The lithium values obtained do not appear to

depend on the degree of alteration of the samples, nor do they appear to be dependent on the mineralogy of the sample. Further investigations are required.

SECTION FOUR

Thorium and Uranium

The distribution trends of the rare radio-active elements thorium and uranium are similar in igneous rocks. Both have characteristic oxidation states of four and their ionic radii are similar (Th^{4+} : 1.02Å, U^{4+} 0.97Å).

These radii are far greater than the radii of any other naturally occurring R^{4+} cations except Ce^{4+}. The highly charged abundant cations such as Si^{4+}, Al^{3+} Fe^{3+} and Ti^{4+} are so much smaller than Th^{4+} and U^{4+} cannot substitute for them in their respective minerals. The radii of Na^{+} and Ca^{2+} are similar to those of Th^{4+} and U^{4+} but the large charge difference prevents appreciable substitution of the radio-active elements in the plagioclase structure. As a result, thorium and uranium are concentrated in acidic rocks, and as a rule, are present in low concentrations in basic and ultrabasic rocks. The trend is therefore similar to potassium, although it is due to different reasons.

The Th/U ratio for the average chondritic and crustal material is approximately 4. The rate of increase of thorium

concentration with differentiation seems, however, to be slightly greater than that of uranium. As a result the Th/U ratio tends to increase in acidic rocks. The ratio may fluctuate quite widely within one rock sequence.

Thorium and uranium are fairly readily accepted into accessory minerals such as allanite, apatite, epidote, monazite, sphene, zircon and members of the thorite group.

However, although much of the thorium and uranium may be incorporated in these accessory minerals, some cannot be accounted for in this way. HURLEY and FAIRBAIRN (1957) have suggested that this may be due to the development of a late phase carrying Th & U, along grain boundaries. This has been demonstrated to be the case for an eclogite from Delegate, Australia by KLEEMAN and LOVERING (1967).

Thorium and uranium were determined in fifty-two kimberlites from eleven occurrences. The range of thorium values obtained is 5.2ppm to 60.5ppm. and that of uranium from 1.0ppm to 7.5ppm. The Th/U ratio varies from 2.58 to 9.95. (Table B 14). The variation of thorium content is greater than that of uranium, resulting in high Th/U ratios.

Several points are worthy of comment.

(1) The kimberlites from the Kimberley area, all major pipe-like occurrences in their surface expression, have similar thorium and

uranium contents and Th/U ratios, which are close to the common average of 4.

(2) The kimberlites from Jagersfontein and Koffiefontein, which are also pipe-like bodies, have similar thorium and uranium contents to those of group (1), and similar Th/U ratios.

(3) The kimberlites from Bellsbank and Southern Fissures, two narrow dykes, are characterised by high thorium and uranium and high Th/U ratios.

(4) Two kimberlite occurrences, intermediate in the character of their surface expression, Roberts Victor and Newlands; show the same features as (3).

The Newlands occurrence consists of four small pipes and a "blow" aligned along a narrow dyke of kimberlite. This dyke continues in the direction of the Frank Smith Mine and is possibly closely related to the Bellsbank dyke. The kimberlite is intermediate between typical micaceous kimberlite such as is found at the Southern Fissures occurrence, and the basaltic kimberlite characteristic of the Kimberley Mines.

The Roberts Victor Mine consists of two large "blows" and two small pipes. It appears to coincide with the line of a persistent dyke. The kimberlite is micaceous.

(5) The high average Th/U ratios from kimberlites from Newlands, Roberts Victor, Bellsbank and Southern Fissures, may indicate that

these are more differentiated than those from the other localities studied. Not much is known about the Southern Fissures occurrence, but the other three are notable for the high amount of eclogite which they contain.

This is interesting as O'HARA and YODER (1967) have suggested that eclogite crystallises at depth from a liquid obtained as a product of partial melting of garnet peridotite. They infer that this liquid has fugitive, (and obviously more differentiated) components, which have a composition similar to the groundmass of kimberlite.

(6) The thorium and uranium content of the kimberlites is not closely related to the potassium content.

General Discussion

TATSUMOTO and LOVERING (1968) report 11.0ppm. Th, 2.69ppm. U and a Th/U ratio of 4.1 for one sample of kimberlite from Kimberley Mine. This is within the spread of values reported here for kimberlite from other closely related pipes.

TATSUMOTO and LOVERING have determined thorium, uranium and lead in this single kimberlite and a single eclogite inclusion from the Roberts Victor Mine. Assuming that the isotopic composition of the lead in the kimberlite of the Roberts Victor Mine is not significantly different from that of the Kimberley Mine, and assuming that the contamination from the host magma is

insignificant, they have deduced that the eclogite xenolith is of "accidental origin". Whether this deduction is correct or not, neither assumption appears to be valid.

The Roberts Victor kimberlites contain much more thorium and uranium than the Kimberley Mine sample, and appear to have a higher Th/U ratio. The two Roberts Victor kimberlites analysed in this study reflect two distinct types. KRV 12 would appear to be more typical than KRV 13, as it is closely related to the other kimberlites.

Six samples of Roberts Victor kimberlite were investigated in this study. Five have a similar appearance to each other, in thin section, and a similar chemical composition. KRV 12 is one of these five. KRV 13 is the sixth kimberlite sample.

The Th/U ratio of KRV 12 is 6.16, very close to the value of 5.9 reported for the Roberts Victor eclogite.

It is suggested that

(1) in view of the evidence presented in this study of the secondary alteration of Roberts Victor eclogite;

(2) as thorium and uranium are not readily accepted into the structure of the primary minerals of eclogite; and

(3) as the kimberlite contains much more thorium and uranium than the nodules, substantial contamination of the nodules may have occurred. The nodule would then be expected to reflect the Th/U ratio of its host kimberlite.

This conclusion is not confirmed by HEIER (1963) who analysed a number of eclogite samples from kimberlites, including several from Roberts Victor. Low Th/U ratios of 0.7-2.5 were reported.

However, the statistical errors quoted for some of these determinations was ± 50%. Further accurate analyses are required to resolve the apparent discrepancy between the determinations of HEIER, TATSUMOTO and LOVERING (1968) and LOVERING and MORGAN (1963).

CHAPTER SEVEN

FURTHER DISCUSSION

It has been shown that ultrabasic xenoliths from kimberlite occurrences have undergone secondary processes which have altered the primary distribution of the trace constituents in particular. The elements most affected are those which cannot substitute readily into the major mineral phases of the nodules, such as potassium, rubidium and cesium. Titanium, phosphorus, uranium, thorium, barium, strontium, zirconium, hafnium and niobium are similar elements in this respect and have been referred to as "incompatible elements" (GREEN and RINGWOOD 1966). In kimberlite, all are present at much higher concentration levels than in average ultrabasic rocks, (DAWSON 1967), and contamination of the xenoliths may occur.

Detailed studies of the element distributions in the individual, pure, primary and secondary mineral phases are required. It may then be possible to estimate both the degree of contamination which a xenolith has undergone and its original composition.

However, in order to do this, it is necessary to identify with certainty the primary and secondary minerals. In addition, it is necessary to bear in mind that kimberlite itself contains diamond and almost certainly has its origin within the upper mantle.

It has been shown in Chapter Six that, assuming there is

only one generation of brown mica in kimberlite xenoliths, all the mica is of secondary origin. However, the assumption is not necessarily correct, and it is possible that there may be primary phlogopite present in some nodules together with one or more generations of secondary phlogopite. If this is so, it may be difficult to differentiate between the varieties as chemical and isotopic homogenisation may have occurred during the secondary processes.

The presence of one or more potassium-bearing minerals in the upper mantle may be necessary to explain the observed characteristics of alkali and tholeitic basalts and to account for the kimberlites themselves.

RINGWOOD and GREEN (1966), OXBURGH (1964), HARRIS (1957, 1967), NICOLLS (1967), YODER and TILLEY (1962) and GREEN (1968) agree that it is difficult, if not impossible, to account for the variations in concentrations of the "incompatible elements" in tholeitic and alkaline basaltic magmas by conventional fractionation processes. Such mechanisms as "zone refining" or "wall rock reactions" have been invoked to explain these features.

Phlogopite would readily accommodate some of these "incompatible elements", such as potassium, rubidium, cesium, titanium and phosphorus. The mineral has been shown to be stable under temperature and pressure conditions which are expected to

prevail down to depths of 150-200km. in the earth. (KUSHIRO et al., 1967). NICOLLS (1967) has shown that the presence of phlogopite in the part of the upper mantle from which alkali basalts are derived would explain their nepheline normative character and the concentration levels of potassium in particular.

OXBURGH (1964) suggested the presence of amphibole as a potassium-bearing mineral in the upper mantle. HART et al. (1967) reported high K/Rb ratios in amphiboles, and the presence of up to 15% amphibole in the peridotite of St. Paul's Rock. KUSHIRO et al. (1967) have suggested that amphibole may be stable at relatively shallow levels in the upper mantle ($\sim$ 100km). They have proposed that amphibole and phlogopite may co-exist in these relatively shallow levels, but that there will be relatively less phlogopite in these regions than at greater depths.

There is evidence that the alkali basaltic magmas originate at greater depths than the tholeites. (YODER and TILLEY, 1962). DICKINSON and HATHERTON (1967) have related increasing potassium content to increasing depth of origin for andesites.

It is interesting to note that if increasing potassium content can be related to increasing depth of origin then the observed K/Rb ratios for tholeitic and alkali basalts may be explained in terms of mineralogical control.

The tholeites, derived from partial melting of the mantle

at relatively shallow depths where amphibole is stable, will have high K/Rb ratios, which reflect the K/Rb ratio of this mineral. Those alkali basalts derived from the greatest depths, will reflect the K/Rb ratio of phlogopite which can be expected to be much lower than that of amphibole. Basalts derived from intermediate depths may have intermediate K/Rb ratios depending on the relative proportions of amphibole and phlogopite present.

Kimberlites, which contain diamonds, and must therefore be derived from very great depths in the upper mantle, are also highly potassic and contain abundant phlogopite. It has been suggested that the potassic nature of kimberlite can best be explained by the assimilation of granitic material into a parental magma, possibly carbonatite (TURNER and VERHOOGEN 1960; DAWSON 1960).

This does not explain the high titanium content of kimberlite. Kimberlite also has an unusually high K/Na ratio which can be accounted for only if the carbonatite assimilates only the orthoclase from the granite.

BAILEY (1964) has shown that the reaction:-

$$K_2O \cdot Al_2O_3 \cdot 6\,SiO_2 + 6\,(CaO \cdot MgO \cdot CO_2) + 2H_2O$$

Orthoclase — Dolomite — Water

$$\rightleftharpoons K_2O \cdot 6MgO \cdot Al_2O_3 \cdot 6\,SiO_2 \cdot 2\,H_2O$$

phlogopite

$$+ 6(CaO \cdot CO_2) + 6\,CO_2$$

Calcite — Carbon dioxide

proceeds to the right at high temperatures. However, the conspicuous lack of thermal metamorphism on the wall rocks of most kimberlites, and the presence of felspathic granulite, granite and arkose containing unaltered potash felspar, implies a low temperature for the final emplacement of kimberlite. Any granitic assimilation must therefore have been carried out at depth. Fresh felspathic inclusions have been observed by the author in the Wesselton Mine and elsewhere, and are recorded by EDWARDS and HOWKINS (1966) at the Williamson Mine, Tanganyika, and by WILLIAMS (1932).

Many of the observed features of kimberlites and its xenoliths can be explained by an alternative hypothesis proposed by O'HARA and YODER (1967), which does not invoke sialic assimilation.

At the depths in the upper mantle at which kimberlite is thought to originate, it is proposed that the undepleted mantle is a four-phase garnet peridotite. When partial melting occurs followed by high pressure fractionation, bimineralic eclogite may form as a crystal accumulate. The fugitive liquid from this fractionation would have the chemical composition of the groundmass of kimberlite.

In support of this idea, it has been experimentally shown that the partial melting of garnet peridotite at 30kb. occurs at a lower temperature than the partial melting of eclogite.

The eclogites, even from one occurrence, show extreme variation in chemical composition. SCHUTTE (1967) reports variations in alumina content from 6.6% to 44.7% in eclogites from the Bellsbank Kimberlite occurrence. WILLIAMS (1932) reports wide variations in the composition of eclogite from Roberts Victor. Banded specimens are known, and the presence of volatile rich fractions along grain boundaries, may represent intercumulitic material as well as secondary alteration products. (Some phlogopite may be introduced in this way.)

The garnet peridotites and the peridotites in general, appear to be far more constant in composition than the eclogites. The peridotites are certainly more common than eclogite, and have been found, by the author, to be present in every kimberlite occurrence in which they have been sought. In most kimberlite the number of peridotite xenoliths present is much greater than the number of eclogites. On the Bultfontein Mine dumps, two hundred and fifty ultrabasic nodules were inspected, and every one was peridotite.

These observations are in accord with the hypothesis of O'HARA and YODER (1967). However, the extremely high concentration of the "incompatible elements" in kimberlite is greater than that in alkali basalts. Minor phases in the mantle are again indicated, and besides phlogopite, these may include ilmenite, apatite and zircon.

SEIFERT and SCHREYER (1968) have shown experimentally that the presence of a potassium and water-rich phase can greatly lower the temperature of initial melting of an olivine-enstatite assemblage, and could lead to the low temperature emplacement of a phlogopite-forsterite rich body in which enstatite is unstable.

This would appear to have a great resemblance to a kimberlite intrusion. It is possible that the presence of a potassium-rich phase in the mantle has an important role to play in the emplacement of kimberlite.

Recent analyses of diopsides from garnet peridotites by electron-probe, confirm that the potassium content of this major phase is low (BOYD 1967). In one case, it is less than 20ppm. MEYER (1968) reports no detectable potassium in a garnet inclusion in a diamond. The olivine and ortho-pyroxene are equally unlikely to contain appreciable potassium, as there are no suitable lattice sites available. The potassium must therefore be accommodated in a minor phase, (e.g. phlogopite).

RICKWOOD, MATHIAS and SIEBERT (1968) have investigated the physical properties, including cell sizes, of garnets from garnet peridotites and eclogites. They have shown that garnets from garnet peridotites have a small range of properties and these reflect a small range in composition, mainly of the pyrope end member.

The garnets from eclogites and pyroxenites show a wide range of physical properties reflecting a wide range in chemical composition.

However, the optical properties of peridotitic garnet do not overlap with those of the eclogitic and pyroxenitic garnets, and this must also apply to their respective chemical compositions.

The data of MEYER (1967, 1968) indicates that the garnet inclusions found in diamonds are similar to eclogite and pyroxenite garnets, and not peridotite garnets. It has been suggested on the basis of synthetic diamond synthesis that diamonds will only grow from solution in nature (GIARDINI and TYDINGS 1962). It is possible that the conditions within the mantle which lead to the partial melting of garnet peridotite, and the subsequent accummulation of eclogite may sometimes also be suitable for the formation of diamonds.

This is a possible explanation for the fact that diamonds have been reported as inclusions in eclogite, (BONNEY 1899, CORSTORPHINE 1907, HOLMES 1937, McGREGOR 1967, SWITZER and MELSON 1968, SIEBERT et al., In Preparation), whilst no occurrence of diamond in the much more common garnet peridotite has been recorded.

The essential linking factor between eclogite and diamond would be that the formation of either must be preceded by the production of a partial melt within the upper mantle. If the

degree of partial melting, the temperature and pressure conditions in the mantle, and the time factor in transport to the surface are suitable, an association of eclogite and diamond may be found. This association could be expected to apply to pyroxenite also.

The ideas and data of several workers, when taken together, therefore, provide a theoretical explanation for an observed feature which had not previously been dealt with satisfactorily. This is, in itself, evidence in favour of the original ideas, including those of O'HARA and YODER (1967) mentioned earlier.

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Late Addendum to the Literature Cited

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E R R A T U M

+ HEIER and ADAMS (1964) has been incorrectly referred to as HEIER (1964).

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