THE KINETICS OF DEGRADATION OF CHLORAMPHENICOL IN SOLUTION
RATE OF DEGRADATION IN NEUTRAL AND ALKALINE SOLUTIONS

BY

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The author wishes to express his sincere appreciation to Dr. T. Higuchi for suggesting the problem and helping in very many ways to guide it to completion.

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INTRODUCTION

Chloramphenicol, the generic name for D-(-)-threo-1-p-nitrophenyl-2-dichloroacetamido-1,3-propanediol (1), is a broad spectrum antibiotic which was first obtained from Streptomyces venezuelae (2). It is unusual in that it is a derivative of nitrobenzene and of dichloroacetic acid, a substance not previously found in a natural product.

The production of chloramphenicol was at first entirely by isolation from culture fluid of Streptomyces venezuelae, but, because of the relatively simple structure of the molecule, attempts were made to synthesize it. This was successfully done in 1949 (3,4,5) and synthetic production is now a very important method for obtaining the drug. Although recently chloramphenicol has been shown to have some undesirable side effects, its activity against infections caused by gram-negative and gram-positive bacilli is considered to be superior to that of any other antibiotic in use at the present (6). For that reason it still enjoys a considerable world-wide use.

This study is concerned with the rate of degradation and elucidation of the mechanism by which this important antibiotic breaks down in aqueous solution.

Despite the fact that chloramphenicol is a relatively stable compound - in fact, the most stable of the known antibiotics - it still undergoes significant degradation in solution. It has been reported (7) that no significant degradation occurs after boiling for five hours with distilled water or after standing for 24 hours at a pH from 2 to 9. But at pH 10.8, it was reported (8) that 87% of the drug was destroyed after standing for 24 hours at 25°C. It was therefore desirable to undertake a kinetic study of its degradation in aqueous solutions so that more effective and efficient methods could be employed for its stabilization.

SCOPE OF THE INVESTIGATION

Chloramphenicol would appear to be susceptible to degradative attack at a number of points in the molecule. Hydrolytic cleavage of the halogens, hydrolysis of the amide, oxidation of the alcoholic groups and reduction of the nitro group all seemed to be processes worthy of study. Preliminary investigations showed, however, that oxidation and reduction were of very minor importance in the breakdown of chloramphenicol except under very severe alkaline conditions. Therefore, attention was directed to the hydrolytic cleavage of the halogens and of the amide bond.

The findings of the present investigation regarding the first aspect of the problem have already been published in the form of a paper. The following is a brief summary of the results.

The over-all reaction of chloramphenicol in water to yield chloride ions is a summation of three reactions: (a) an uncatalyzed hydrolysis; (b) a hydrolytic cleavage of the amide bond yielding dichloroacetate ions which in turn yield chloride ions; and (c) a hydroxyl ion catalyzed hydrolysis. The uncatalyzed reaction is the principal one responsible for

breakdown below a pH of 6. At a higher pH, the catalyzed hydrolysis yielding chloride ions and the breakdown of the amide are the predominant reactions, the latter being wholly responsible at very high hydroxyl ion concentrations. It is believed that the cleavage of the amide directly influences the amount of chloride production by, first, decreasing the amount of chloramphenicol available for hydrolysis of chloride ions, and, second, by increasing the amount of dichloroacetate ions which break down into chloride ions.

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I. A Study of the Rate of Formation of Chloride Ion in Aqueous Media

In carrying out the second assignment, it was necessary to develop a new assay method for chloramphenicol per se, since the only assay procedure available at that time for determination of chloramphenicol was an approximate microbiological method based on turbidimetry. This method gave results reproducible to within only 5% and was not precise enough for the purpose at hand. A new microbiological method has been devised, based on the uptake of oxygen by Escherichia coli, using a manometric technique. This method has the advantage of being less time-consuming than the turbidimetric method, but it too is not capable of giving results of greater reproducibility than 5%. Therefore, a second method has been developed involving the separation of chloramphenicol by means of partition chromatography and subsequent determination spectrophotometrically. This procedure offers the advantage not only of being fairly rapid, but also of a reproducibility to within 0.1%. It has been, therefore, the assay method of choice whenever determinations of the amount of chloramphenicol present are desired.

COMPARISON OF A MICROBIOLOGICAL AND A CHROMATOGRAPHIC ASSAY METHOD FOR CHLORAMPHENICOL

After the development of a more efficient and precise assay method for chloramphenicol, it was possible to undertake a study of the rate of hydrolytic cleavage of the amide bond in both neutral and basic media. The results obtained show that: (a) the over-all degradation rate of chloramphenicol in aqueous solution was first order with respect to the antibiotic over a wide range of hydrogen ion concentration; (b) the rate of chloramphenicol degradation is independent of the ionic strength of the medium and largely independent of the hydrogen ion concentration within the pH range 2-7; and (c) chloramphenicol deterioration is catalyzed by species which are general acids and bases. These results have been published as a paper, a reprint of which follows.

THE KINETICS OF DEGRADATION OF CHLORAMPHENICOL IN SOLUTION

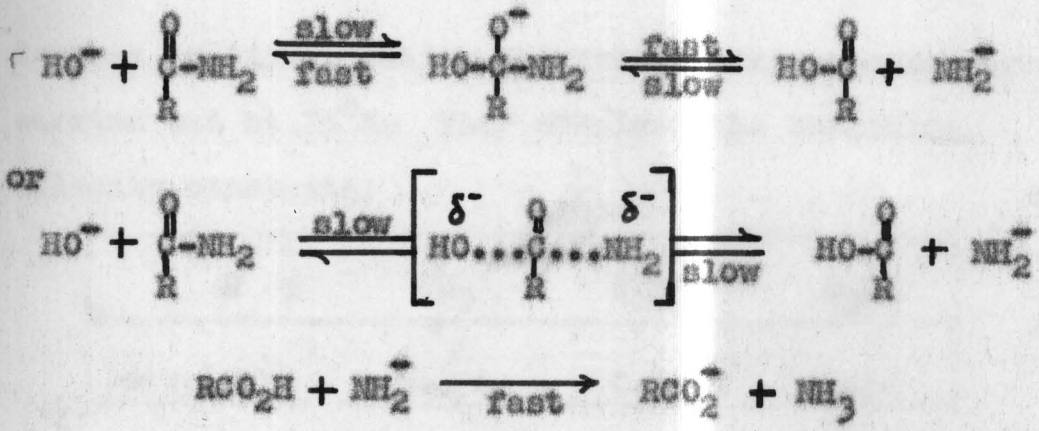
II. Over-all Disappearance Rate from Buffered Solutions

PREVIOUS WORK ON THE ALKALINE HYDROLYSIS OF AMIDES

In order to understand the possible mechanisms operating in the hydrolytic cleavage of the amide bond in chloramphenicol, it is desirable to study past work done on alkaline hydrolysis of amides. Although information is not too extensive, certain basic inferences can be gained.

The hydrolysis of amides is catalyzed by both hydrogen and hydroxide ions, but more effectively by the latter. The variation in the velocity of hydrolysis of benzamide with change in the pH of the solution has been studied by Bolin (9) who found that the rate constant at 20°C. increased from 0.061×10^{-4} to 10.0×10^{-4} as the pH increased from 9.42 to 11.22; the effect on the rate constant of hydrogen ions was much smaller.

The structure of acid amides, which contain a carbonyl group for the attack of hydroxyl ion, and a weakly basic amino group at which a protophilic attack can occur, suggests that the mechanisms of acid and base catalysis postulated for esters should also apply. Since the velocity of hydrolysis of amides in dilute aqueous solutions of bases is dependent both on the concentration of the amide and of hydroxyl ions, the base-catalyzed reaction may be formulated as follows (10):



Complications introduced by steric and other factors in the effect of substitution on the velocity of esters apply equally well to amides. Reliable data, however, of the effects of such substitution are much more scanty in the case of amide hydrolysis, but that which is available supports the above views. In general, introduction of electron-attracting substituents into the R group will favor alkaline hydrolysis, the effect of electron-repelling substituents being the reverse.

Crocker and Lowe (11) investigated the hydrolysis of aliphatic acid amides $RCONH_2$ in dilute sodium hydroxide at 63.2°C. They obtained the following velocity constants:

R =	H	CH_3	C_2H_5	C_3H_7	C_4H_9
k (min^{-1})	1.776	0.047	0.041	0.017	0.016

Peskoff and Meyer (12) studied essentially the same amides, but they used 1 N. potassium hydroxide

instead of dilute sodium hydroxide. Experiments were carried out at 25°C. They obtained the following velocity constants:

R =	CH_3	C_2H_5	C_3H_7
k (min^{-1})	0.134	0.135	0.052

They obtained substantially the same rate constants when they used ½ N. potassium hydroxide.

Willems and Bruylants (13) found the following rate constants for $RCONH_2$ using 1/20 N. sodium hydroxide and 1/20 N. amide.

k = liter $mole^{-1}$ sec^{-1} x 10^4

R \ T	65°C.	75°C.	85°C.
CH_3	6.34	11.3	20.6
C_2H_5	5.24	9.98	17.7
C_3H_7	2.34	4.34.	6.43
C_4H_9	2.00	3.23	6.59

They obtained 14.8 kcal. as the heat of activation for the reaction between the amide and hydroxyl ion, and found that it was fairly constant for homologous series at a given pH.

A study of the substituent effects in acid and base hydrolyses of aromatic amides was recently made

by Meloche and Laidler (14). Their data is given below for alkaline hydrolysis using 0.025 M. sodium hydroxide.

rate constants $k \times 10^6$ sec^{-1}

Amide \ T	100.1°	80.3°	64.5°	52.8°
p-nitrobenzamide	2270	582	217	93.9
p-chlorobenzamide	502	125	39.7	16.1
benzamide	338	76.4	23.9	8.54
p-methyl benzamide	188	47.5	13.1	4.66
o-methyl benzamide	13.5	2.37		

They found that changes in the rate are affected mainly by changes in the activation energy. In alkaline hydrolysis, electron-attracting groups lower the activation energy, while electron-repelling groups raise it. This is in agreement with previous work done by Reid (15,16) who investigated the effects of aromatic substituents on the rates of hydrolysis of substituted benzamides with dilute aqueous barium hydroxide at 100°C. His data is summarized below:

	H	p-NO_2	p-Cl	m-Br	p-Br	m-I	p-I
10^4 k	994	6300	1800	2820	1800	2450	1590

	p-OMe	p-Me
10^4 k	462	623

His figures show that electron-attracting meta- and para-substituents do accelerate, and electron-releasing substituents retard hydrolysis. They also show that ortho substituents retard the reaction independently of their polarity, in agreement with the assumption of a strong steric effect.

The final phase of this work which has not been published deals with the degradation of the drug in alkaline solutions. This is dealt with in greater detail in the following sections.

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III. REACTION RATE IN ALKALINE SOLUTIONS

INTRODUCTION

In earlier publications (17, 18, 19) it was shown that chloramphenicol breaks down in neutral and acidic aqueous solutions by hydrolysis at the amide linkage and by hydrolytic cleavage of the halogens. In the pH regions studied (pH 2-8) it appears that the former is the important degradative pathway and is subject to an uncatalyzed reaction as well as to hydrogen ion and general acid catalysis. Dehalogenation on the other hand is both uncatalyzed and hydroxyl ion catalyzed, but is relatively insignificant up to a pH of 7.

This communication presents the results of an extension of these studies to more alkaline systems. It is shown that the breakdown reaction in basic solutions is relatively complex and presents several anomalies which are difficult to interpret.

CORRELATION OF RATE OF CHLORIDE ION PRODUCTION TO RATE OF AMIDE CLEAVAGE

Theoretical Considerations

The over-all reaction of chloramphenicol in water to yield chloride ions is a summation of three reactions: (a) an uncatalyzed hydrolysis; (b) a hydroxyl ion catalyzed hydrolysis; and (c) a hydrolytic cleavage of the amide bond yielding dichloroacetate ions which in turn yield chloride ions (17). The uncatalyzed reaction is the principal one responsible for breakdown below a pH of 6. At a higher pH, the catalyzed hydrolysis yielding chloride ions and the breakdown of the amide are the predominant reactions, the latter being wholly responsible at very high hydroxyl ion concentrations. It is believed that the cleavage of the amide directly influences the amount of chloride production by, first, decreasing the amount of chloramphenicol available for hydrolysis of chloride ions, and, second, by increasing the amount of dichloroacetate ions which break down into chloride ions.

The rate of chloride ion formation and the rate of amide cleavage are both dependent on the chloramphenicol concentration. Hence, a relationship based on the above hypothesis can be derived mathematically

whereby the rate of amide cleavage is expressed solely in terms of the rate of chloride ion production. This may be treated as follows:

$$\frac{d\,(CM)}{dt} \propto (CM) \tag{1}$$

or

$$\frac{d\,(CM)}{dt} = -k_1\,(CM) \tag{2}$$

where (CM) = concentration of chloramphenicol at any given time, t. By integrating, and letting $(CM)_o$ = concentration of chloramphenicol at t = 0, then:

$$\ln \frac{(CM)}{(CM)_o} = -k_1 t \tag{3}$$

or

$$\ln (CM) = \ln (CM)_o - k_1 t \tag{4}$$

Since the disappearance of chloramphenicol is proportional to the amount of chloride ion formation, we may write

$$\frac{d\,(Cl^-)}{dt} = 2\,k_2\,(CM) \tag{5}$$

(It is assumed that two moles of chloride ions are formed from one mole of chloramphenicol). Then substituting equation (4) into equation (5), we have

$$\frac{d\,(Cl^-)}{dt} = 2\,k_2\,e^{\ln (CM)_o - k_1 t} \tag{6}$$

Integrating, we obtain

$$(Cl^-) = -2\,\frac{k_2}{k_1}\,e^{\ln (CM)_o - k_1 t} + A \tag{7}$$

At $t = 0$, $(Cl^-) = 0$; then

$$A = 2\frac{k_2}{k_1} e^{\ln (CM)_o} \tag{8}$$

Therefore:

$$(Cl^-) = 2\frac{k_2}{k_1}\left[e^{\ln (CM)_o} - e^{\ln (CM)_o - k_1 t}\right] \tag{9}$$

or

$$(Cl^-) = 2\frac{k_2}{k_1} e^{\ln (CM)_o}\left[1 - e^{-k_1 t}\right] \tag{10}$$

Inspection of equation (10) shows that as t approaches infinity,

$$(Cl^-) = 2\frac{k_2}{k_1} e^{\ln (CM)_o} \tag{11}$$

Rearranging equation (10) produces

$$\frac{(Cl^-)}{2\frac{k_2}{k_1} e^{\ln (CM)_o}} = 1 - e^{-k_1 t} \tag{12}$$

Setting equation (11) equal to $(Cl^-)_\infty$, and substituting into equation (12), we get

$$\frac{(Cl^-)}{(Cl^-)_\infty} = 1 - e^{-k_1 t} \tag{13}$$

By rearranging and taking logarithms, we have

$$-\ln\left[1 - \frac{(Cl^-)}{(Cl^-)_\infty}\right] = k_1 t \tag{14}$$

Thus by obtaining the amount of chloride ions formed when t approaches infinity, we can get a rate constant which should be the same as the rate constant obtained by measuring the rate of disappearance of chloramphenicol itself. By choosing a suitable pH and temperature, where the breakdown of chloramphenicol to the amine is reasonably fast, the value of $(Cl^-)_\infty$ can be readily obtained, since chloride ion formation will cease once the chloramphenicol has been completely degraded to the amine. Kunze showed (20) that chloride ions are formed very slowly from dichloroacetate ions so that complications from this reaction are very small.

Results

The validity of the hypothesis and of equation (14) above could be checked readily by plotting $-\ln\left[1 - \frac{(Cl^-)}{(Cl^-)_\infty}\right]$ against time. If a straight line was obtained for a given pH and temperature, then the equation could be said to represent the actual situation. This has been done in Fig. 1, where all the curves appear to be sensibly linear. Since the total amount of chloride ions available is about 10 millimoles, and the amount titrated varies from 1% to 5% of that, it would not be expected that any great degree of accuracy could be attained. By the time that 5% of the total chloride has been hydrolyzed, the chloramphenicol molecules themselves have been split at the amide linkage. The remainder of the chloride ions which can then be titrated represents almost totally the hydrolysis rate of the chloride ions coming from dichloroacetate ions. Evidence for this is given in Table I which shows how chloride formation approaches a limiting value.

The rate constant for hydrolysis of the dichloroacetate ions is slow (0.0001 hr^{-1}). Nevertheless, its value has been subtracted from the actual rate constant obtained experimentally, so that only that portion of chloride which comes from the intact molecule of

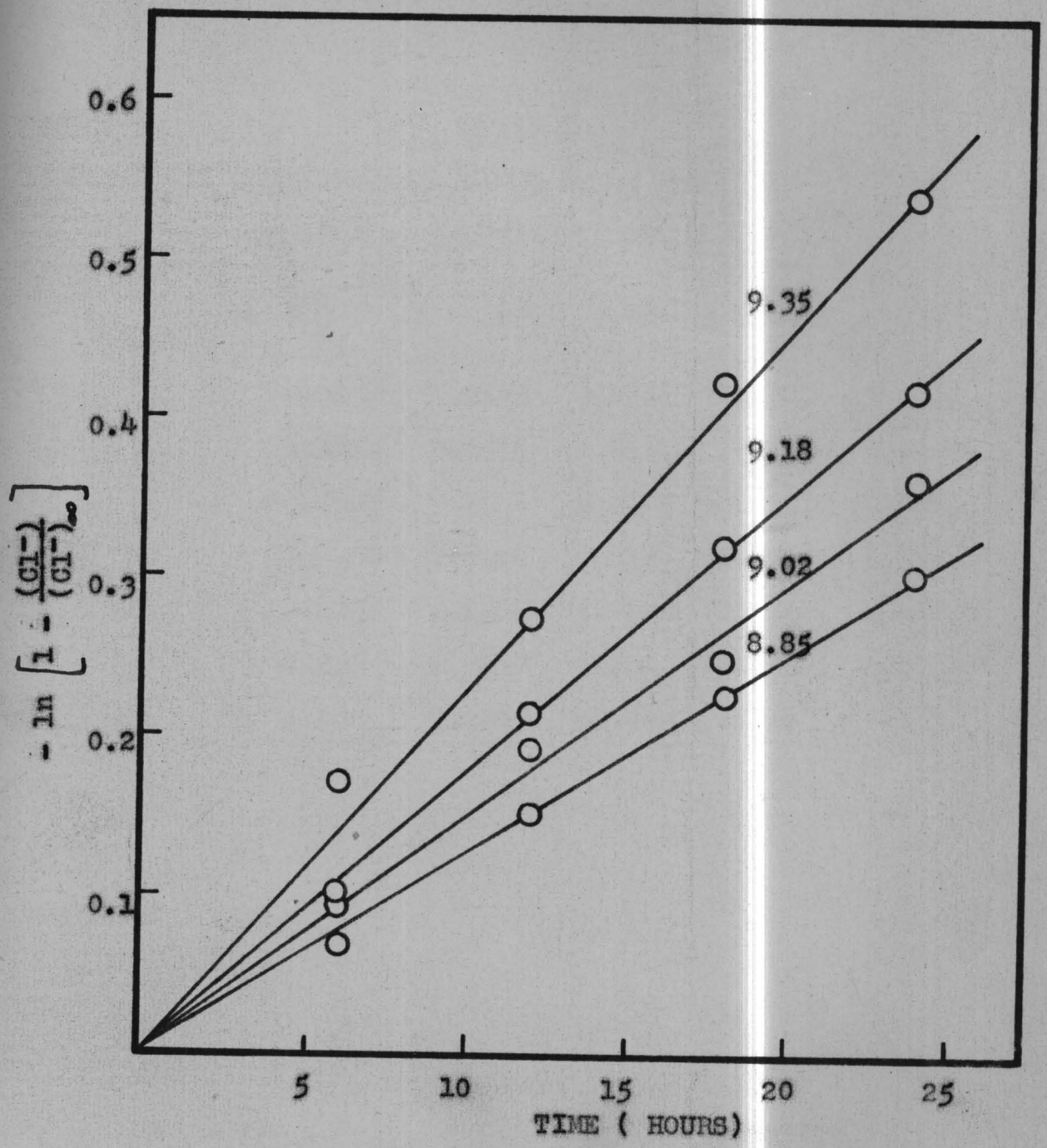


Fig 1. Plots showing the straight line relationships of the function $-\ln\left[1-\frac{(Cl^-)}{(Cl^-)_\infty}\right]$ against time, at pH values indicated. The slope of each curve is equal to the rate constant for the over-all degradation of chloramphenicol.

TABLE I

EQUIVALENTS OF CHLORIDE ION PER MOLE OF CHLORAMPHENICOL . . .

Hours \ pH	8.70	8.85	9.02	9.18	9.34
3	0.0105	0.0105	0.0157	0.0183	0.0314
6	0.0209	0.0235	0.0314	0.0366	0.0470
9	0.0392	0.0340	0.0392	0.0523	0.0680
12	0.0418	0.0470	0.0549	0.0654	0.0812
23	0.0706	0.0680	0.0863	0.105	0.118
130	0.139	0.149	0.162	0.175	0.183
216	0.178	0.186	0.199	0.209	0.215

chloramphenicol has been included in the calculations.

Table II gives the rate constants for the disappearance of chloramphenicol. k_1 represents the values obtained from Fig. 1, and k_1^* represents the values interpolated from Fig. 2, which is a plot showing the rate of disappearance of chloramphenicol at the same pH values as were used in the chloride ion determinations. The two sets of values are quite similar, at least as to order of magnitude, and it is concluded that the equations presented above represent the general over-all picture in the pH range used.

TABLE II

COMPARISON OF RATE CONSTANTS OBTAINED FROM CHLORIDE ION DETERMINATIONS (k_1) AND THOSE OBTAINED FROM DISAPPEARANCE OF CHLORAMPHENICOL (k_1^*)

pH	k_1	k_1^*
8.70	0.025	0.022
8.85	0.026	0.028
9.02	0.030	0.033
9.18	0.036	0.043
9.35	0.047	0.054

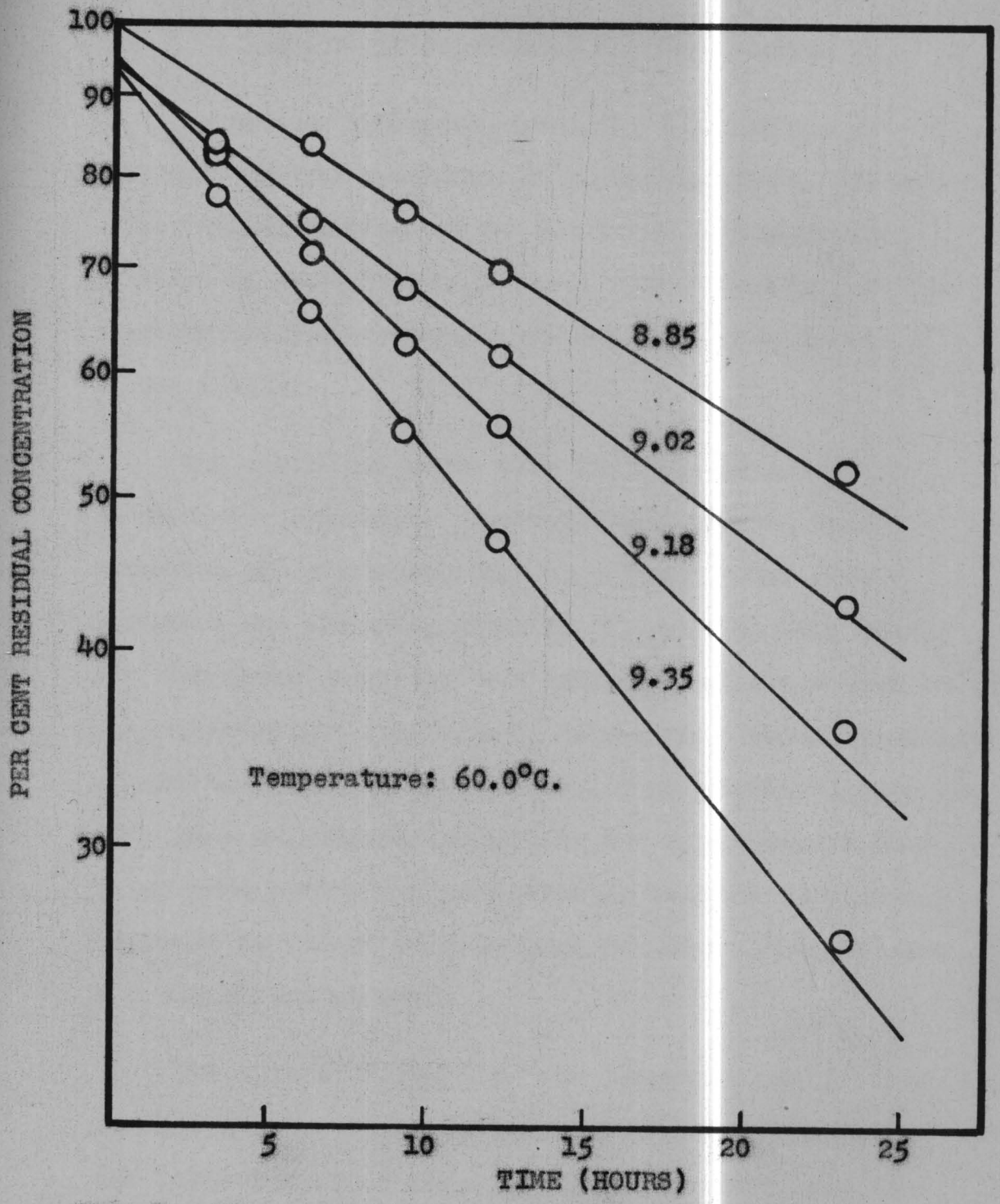


Fig. 2. Plots showing the over-all degradation of chloramphenicol in 0.25 molar carbonate buffers at pH as indicated.

DEPENDENCY OF RATE IN ALKALINE SOLUTIONS

Effect of Hydroxyl Ion Concentration

As the pH increases, there is a significant increase in the breakdown of chloramphenicol. To determine this dependency on hydroxyl ion concentration, systems of higher pH were used. Unfortunately, satisfactory buffering systems are not available in the pH range 10-12.

The use of an amine plus its salt seemed to be a promising approach. Consequently, several were titrated potentiometrically to determine the most suitable one for our purposes. Piperidine fell within the desirable range and its buffering action seemed to be satisfactory. Therefore, it was used in all studies within the pH range 10-12. Fig. 3 shows the disappearance rate of chloramphenicol in 0.2 molar piperidine-piperidine hydrochloride buffer at various pH values. The rate is linear with respect to chloramphenicol for all the pH values used.

The effect of hydroxyl ion concentration is shown in Fig. 4 where the logarithms of the rate constants are plotted against pOH. The resulting straight line has a slope of one. Thus the breakdown of chloramphenicol is first order with respect to hydroxyl ion

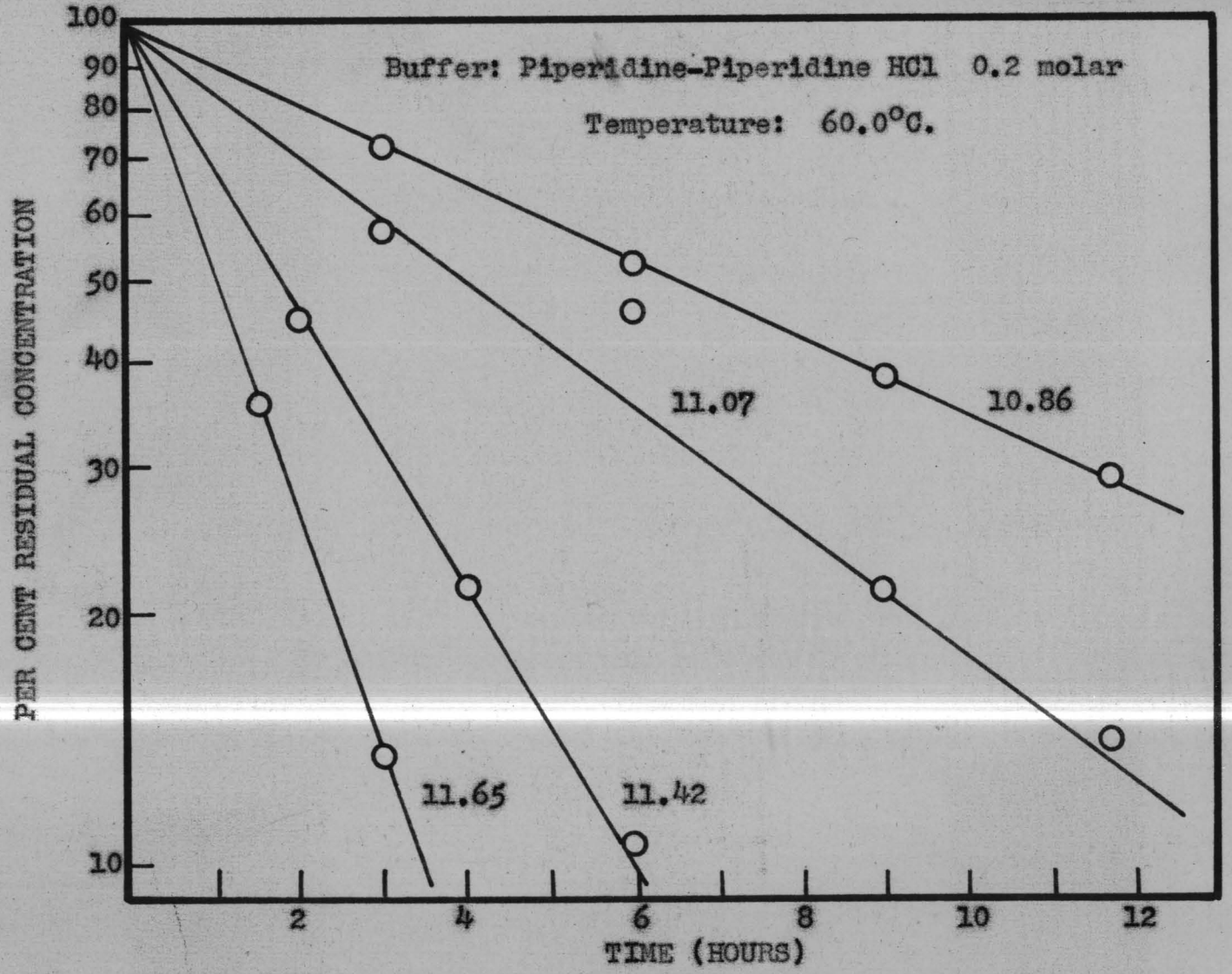


Fig. 3. Plots showing the over-all first order character of chloramphenicol degradation at pH values as indicated.

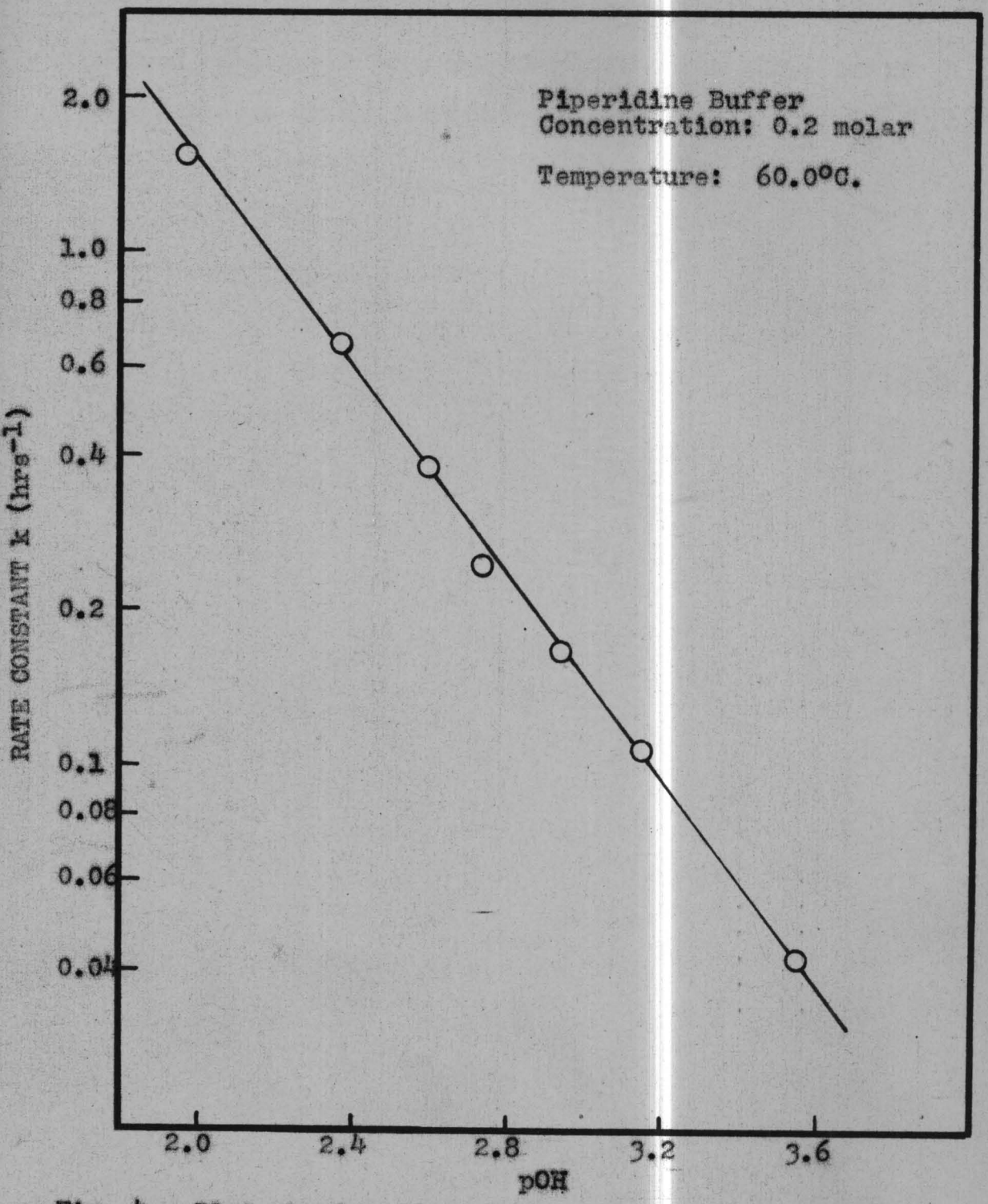


Fig. 4. Plot showing first order dependency of breakdown of chloramphenicol on hydroxyl ion concentration.

concentration. This is to be expected since previous investigations (11-16) of the hydrolytic cleavage of amides with bases have consistently shown first order dependencies on hydroxyl ion concentration.

Dependency of Rate on Buffer Concentration

It was desirable, further, to determine how much of the degradation was due to the hydroxyl ion concentration and how much to the buffer used. Accordingly, several systems were followed using different concentrations of buffer, but adjusting the pH to the same value. Figs. 5 and 6 show that the buffer concentration has a decided influence on the degradation of chloramphenicol. These results are in agreement with those of Trolle-Lassen (21). Fig. 7 shows the extent of the catalytic action of the buffer. As the pH increases, the catalytic effect of increased buffer concentration also increases. However, a point is soon reached (going up the pH scale) where the effect of hydroxyl ion catalysis completely overwhelms the catalytic effect of the buffer. Thus at a pH of 12.05, the same rate is obtained for buffer concentrations varying from 0.2 to 0.6 molar.

By using the rate constants at zero concentration of buffer obtained from Figs. 5 and 6, and combining them with the rate constant obtained at pH 12.05 where the buffer concentration has no influence, a plot may be obtained showing the actual dependence of the disappearance rate on hydroxyl ion itself. Such a graph

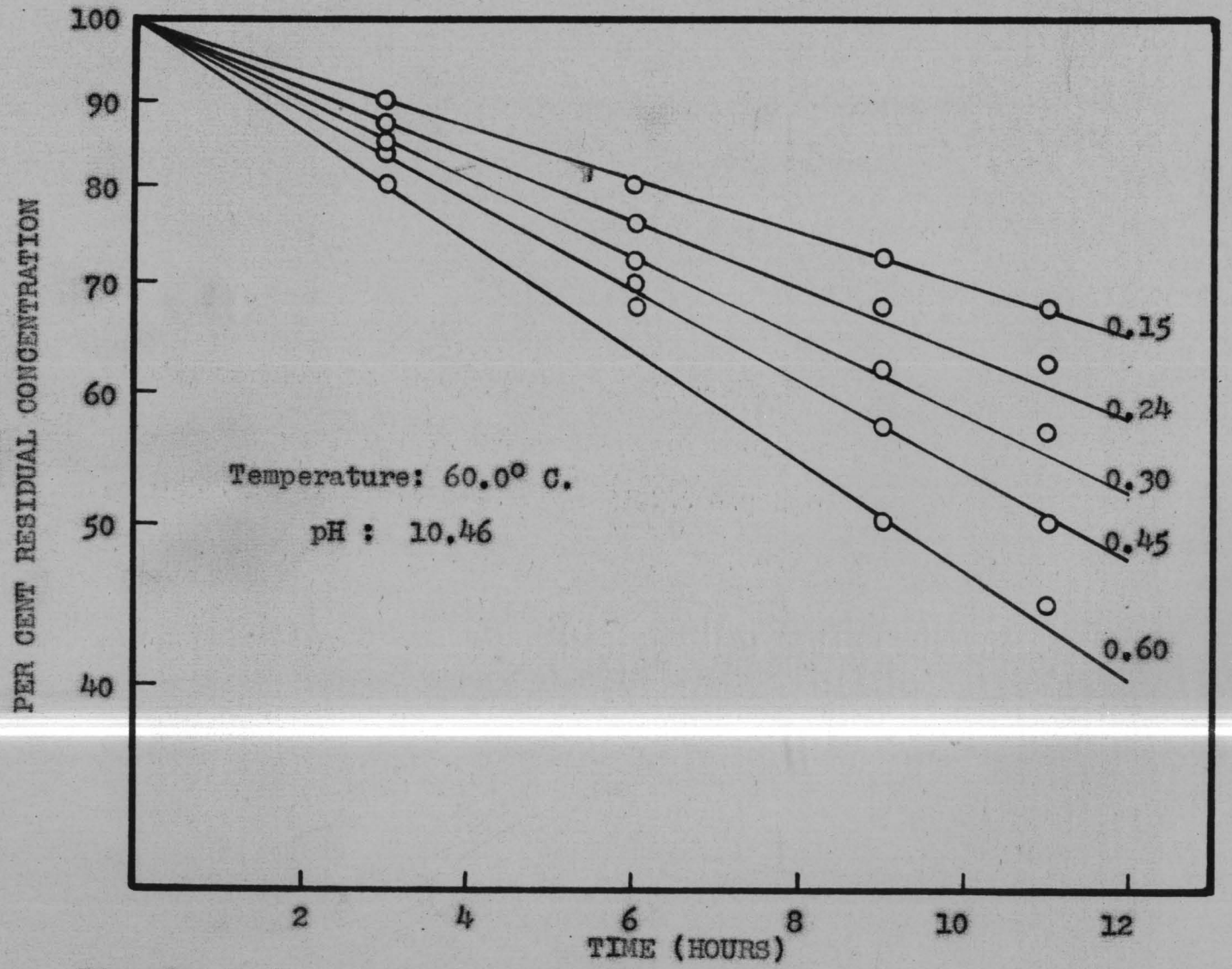
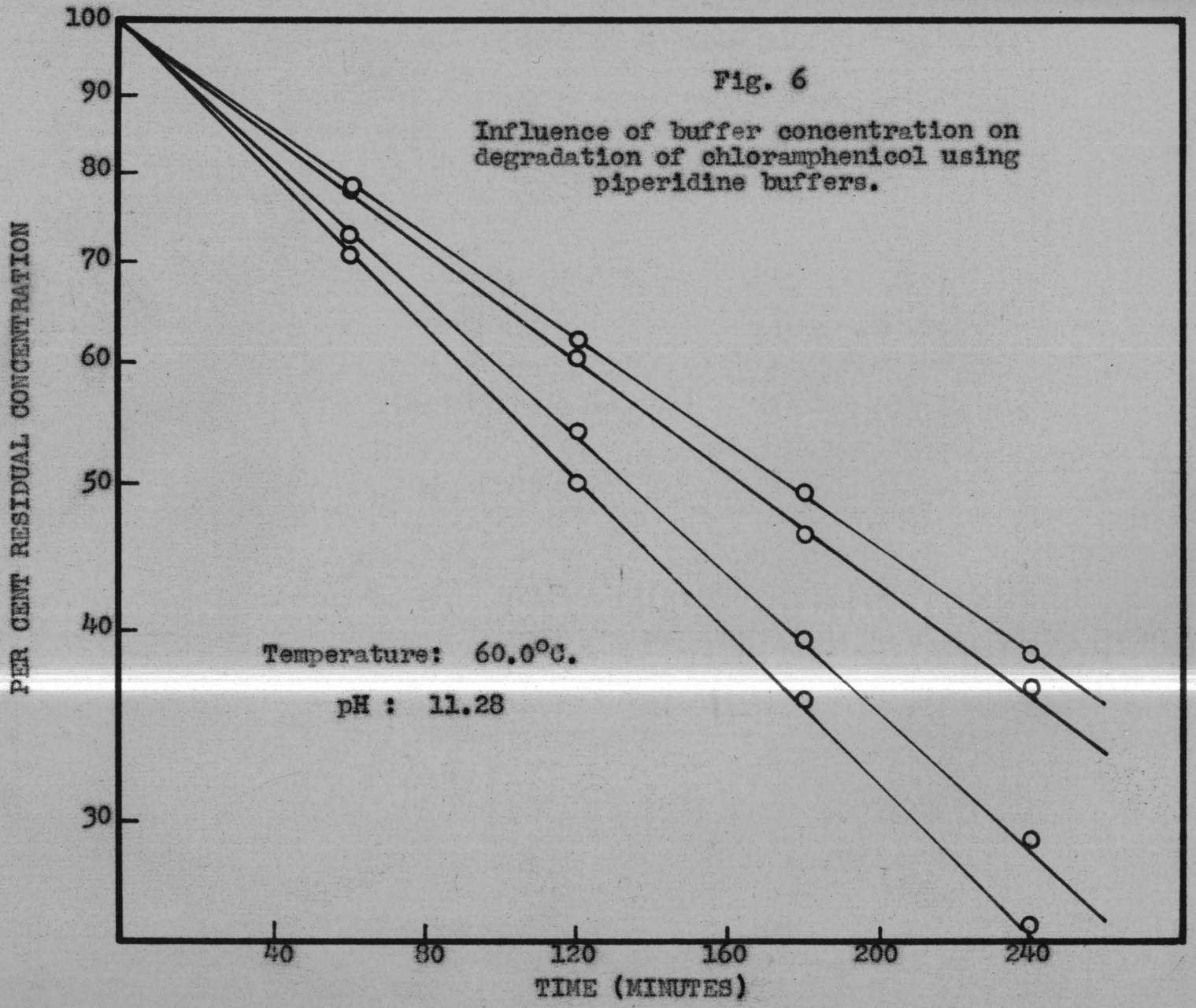


Fig. 5. Influence of buffer concentration on chloramphenicol degradation. Molarities of piperidine buffers are as indicated.

Fig. 6

Influence of buffer concentration on degradation of chloramphenicol using piperidine buffers.



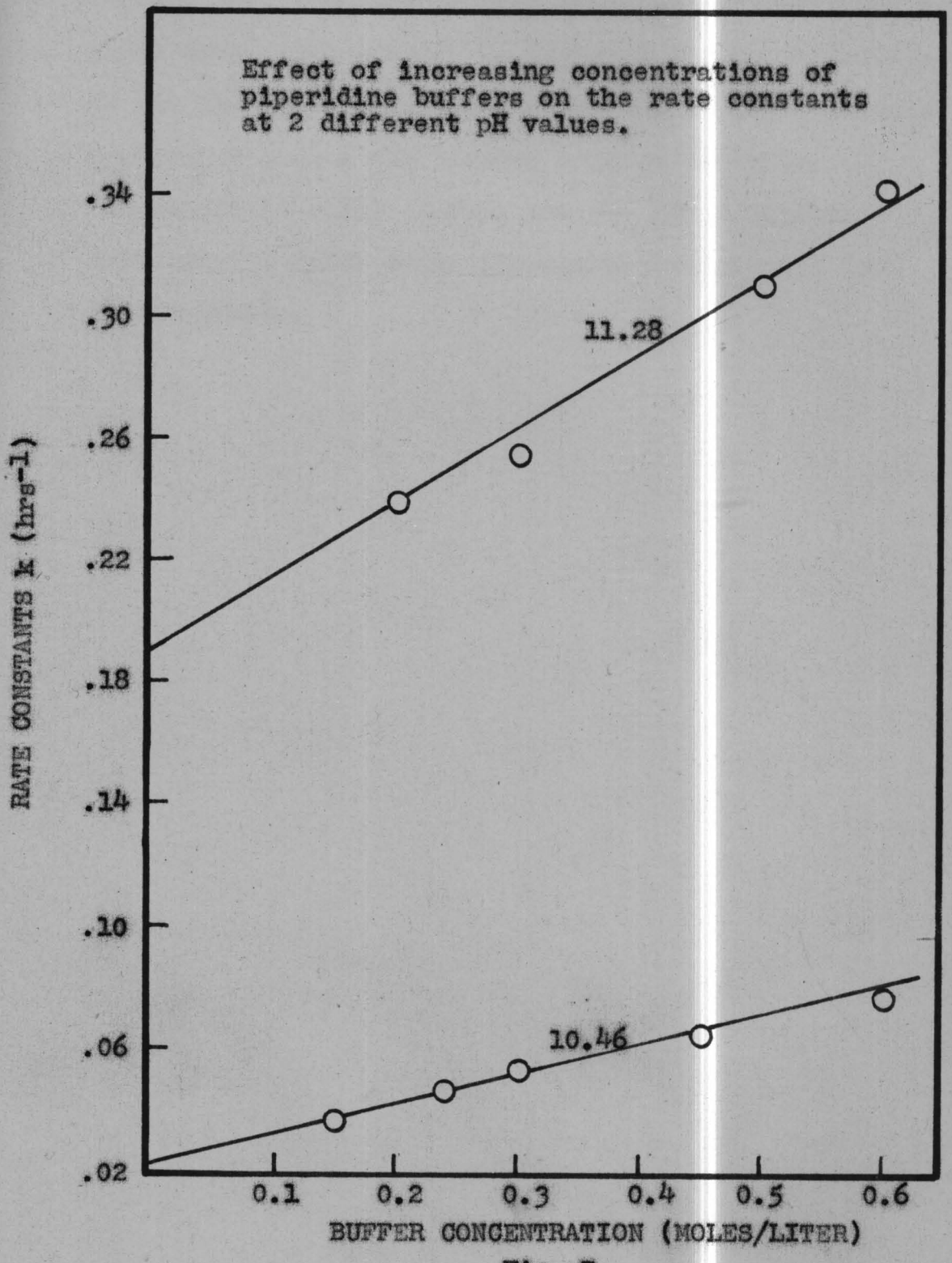

Effect of increasing concentrations of piperidine buffers on the rate constants at 2 different pH values.
RATE CONSTANTS K (hrs-1)
.34
.30
.26
.22
.18
.14
.10
.06
.02
11.28
10.46
0.1
0.2
0.3
0.4
0.5
0.6
BUFFER CONCENTRATION (MOLES/LITER)

Fig. 7

plotted is shown in Fig. 8. Here the slope is linear, but the reaction rate is just slightly greater than first order with respect to hydroxyl ion concentration. If all the reaction rates obtained at a finite concentration of buffer are plotted against pOH (Fig. 4), the result is still linear; but the slope is one, indicating a first order dependency on hydroxyl ion concentration.

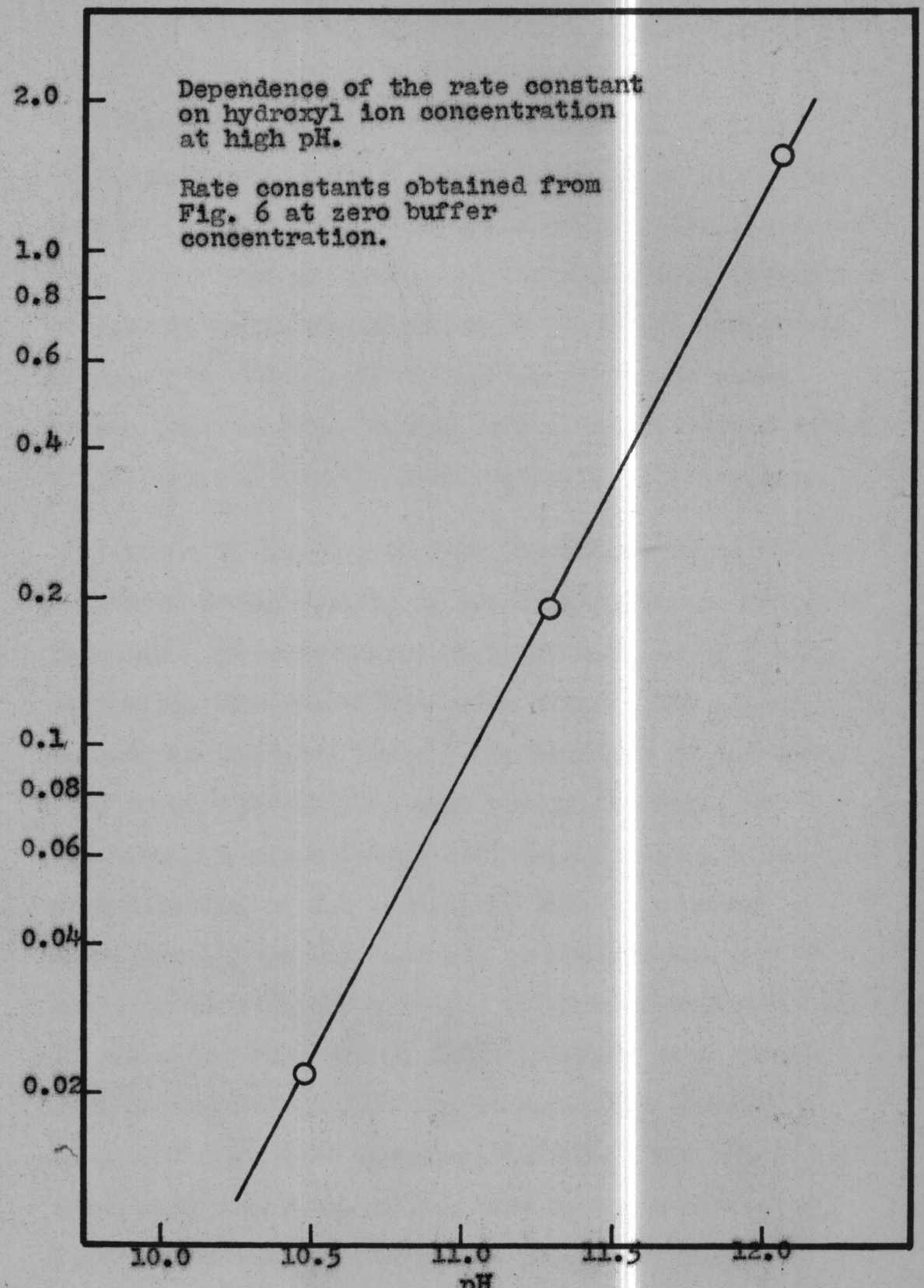

Dependence of the rate constant on hydroxyl ion concentration at high pH.
Rate constants obtained from Fig. 6 at zero buffer concentration.
2.0
1.0
0.8
0.6
0.4
0.2
0.1
0.08
0.06
0.04
0.02
10.0
10.5
11.0
11.5
12.0
pH

Fig. 8

Effect of Hydroxyl Ions in Highly Alkaline Solutions

The concentration of chloramphenicol in these experiments was about 5 millimoles. Hence it was not feasible to use the strong bases as degrading media at a pH lower than 12 because of the significant changes in the hydroxyl concentration at these low concentrations. But above a pH of 12, use of strong bases became practicable. Barium hydroxide was chosen because of its greater freedom from carbonate interference.

Figs. 9, 10, and 11 show the effect of barium hydroxide concentration on the degradation of chloramphenicol. At concentrations above 0.02 molar barium hydroxide, the graphs are quite linear, but slight deviations occur at lower concentrations of the base. This would naturally be expected if the rate was dependent on the hydroxyl ion concentration, since the concentration of the base would then be changed significantly as the reaction progresses and not be constant as would be the case at higher concentrations. To allow for this error, initial values were given more importance in plotting to determine the rate constants than were the final values. This was especially important in the case of the reaction at higher temperature, since high concentrations of

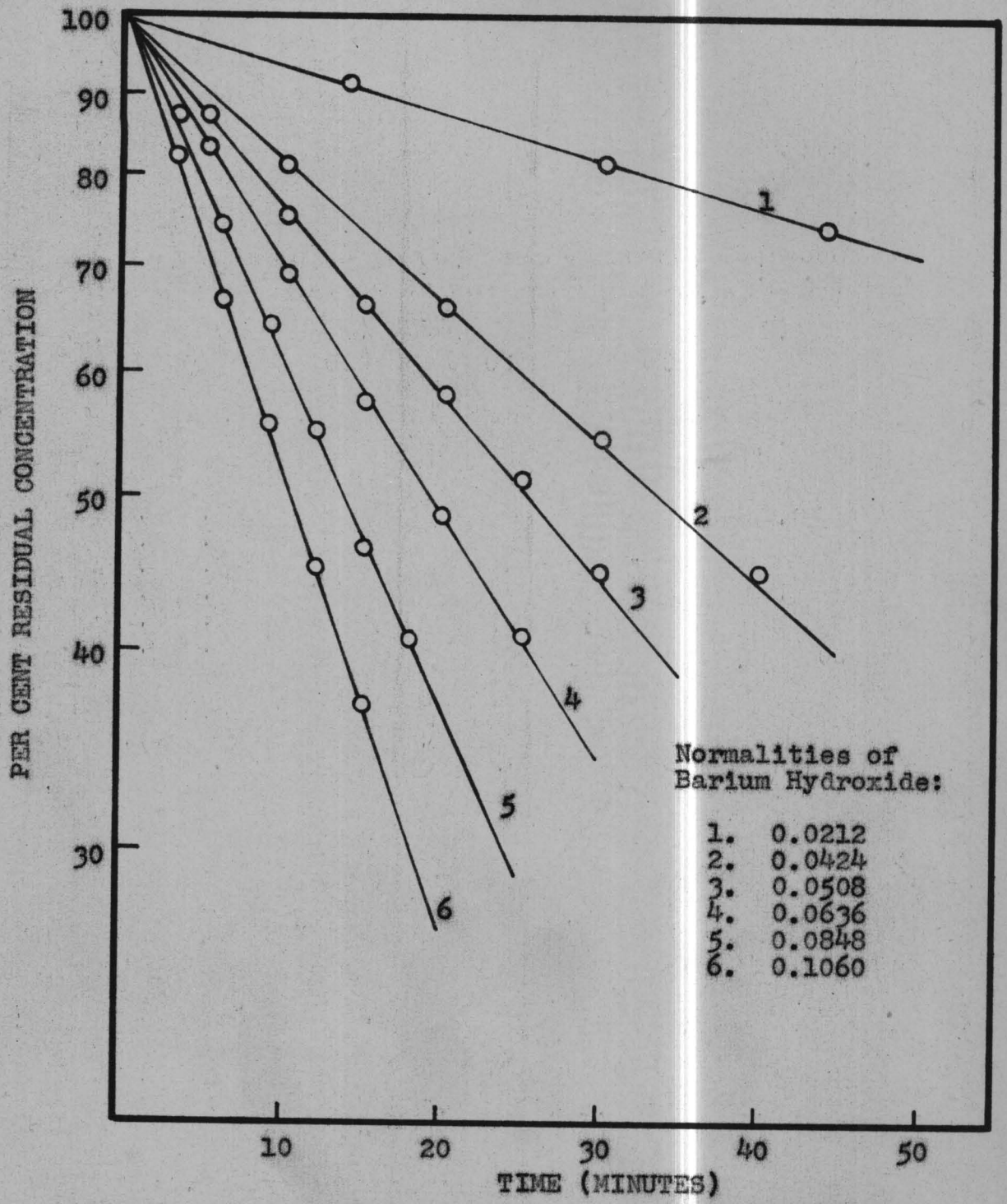
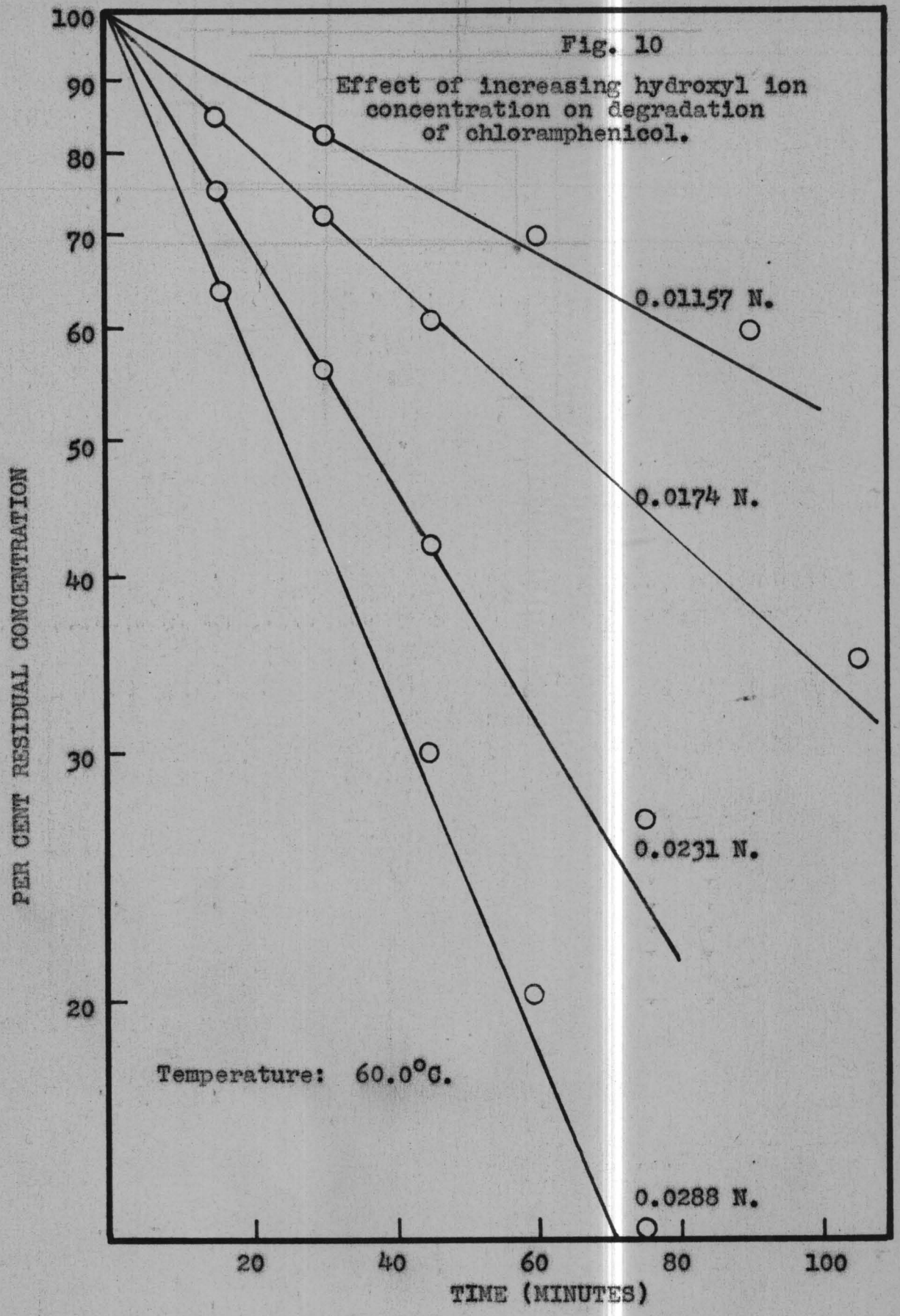


Fig. 9. Plots showing effect of increasing hydroxyl ion concentration on degradation of chloramphenicol at 25.0°C.

Fig. 10

Effect of increasing hydroxyl ion concentration on degradation of chloramphenicol.



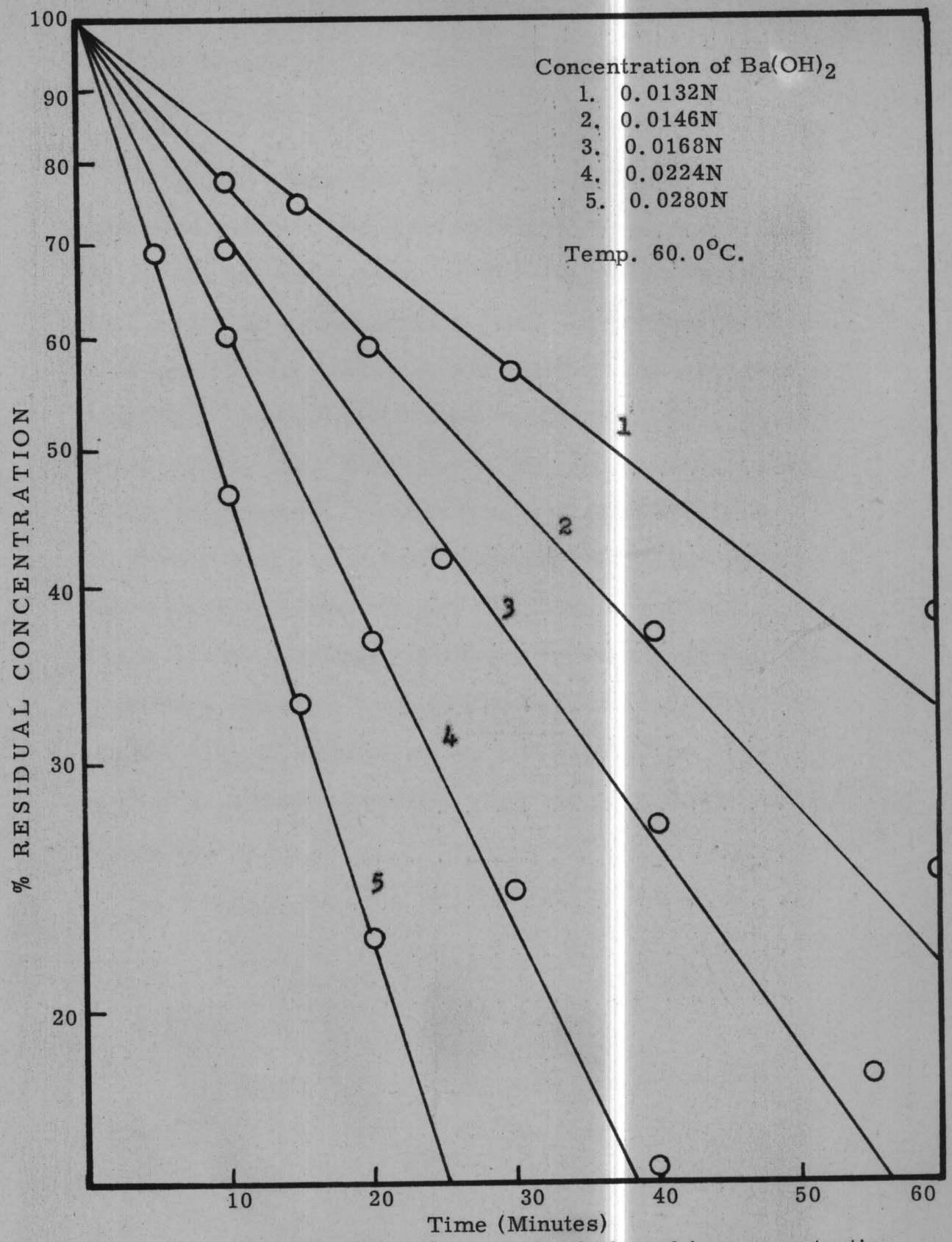


Fig. 11. Plots showing effect of increasing hydroxyl ion concentration on degradation of chloramphenicol at 60.0°C.

the base could not be used because of the resulting rapidity of the reaction.

Fig. 12 shows the linear dependency of the reaction rate on the hydroxyl ion concentration for all 3 temperatures used. The rate is zero at about 0.01 moles of hydroxyl ion. This would seem to indicate that 5 millimoles of chloramphenicol are reacting initially with 10 millimoles of hydroxyl ion; or, in other words, that there is an initial reaction second order with respect to hydroxyl ion concentration. To verify this, chloramphenicol was titrated potentiometrically and conductometrically with barium hydroxide. There was no evidence, however, of such a second order reaction. As yet, we have found no satisfactory explanation of this anomaly, but it will be noted that a similar anomaly occurred with the piperidine buffers (Fig. 8).

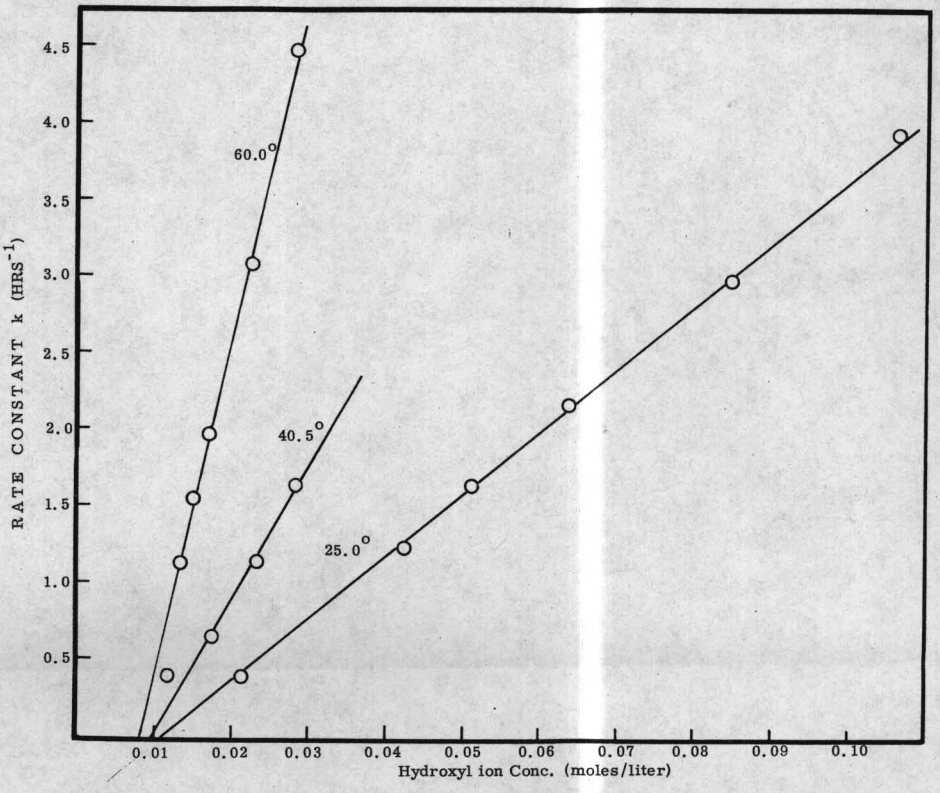


Fig. 12. RATE OF CHLORAMPHENICOL BREAKDOWN IN BARIUM HYDROXIDE SOLUTION AT SEVERAL TEMPERATURES.

Temperature Dependency of the Hydroxyl Ion Catalyzed Reaction

For the barium hydroxide catalyzed reaction, the dependency is linear within the range 25° to 60°C. (Fig. 13). The heat of activation is 10.5 kcal., seemingly a rather low value; but not too unexpected from the evidence in Fig. 12 of the very great sensitivity of the reaction rate to temperature.

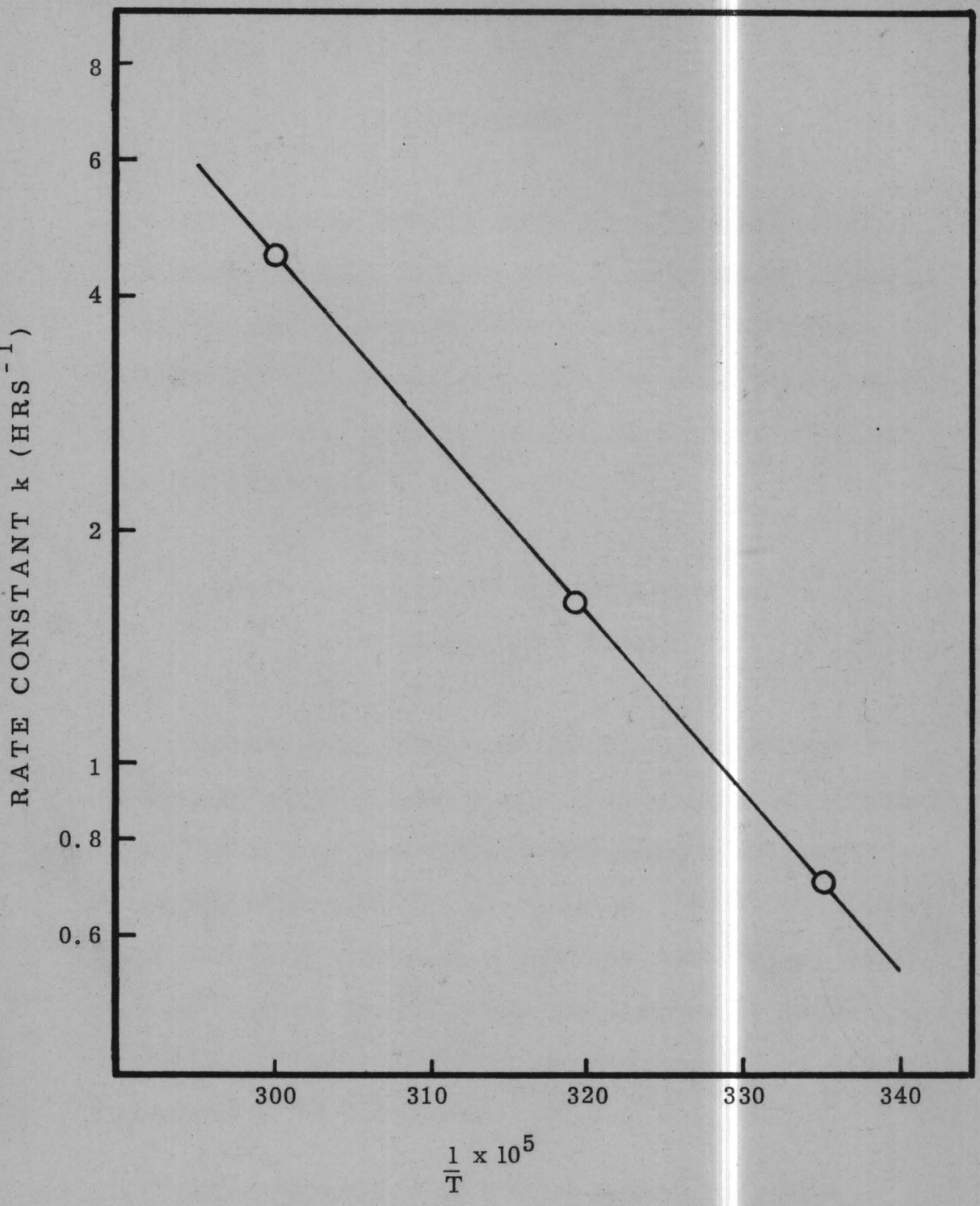


Fig. 13. ARRHENIUS TYPE PLOT OF THE TEMPERATURE DEPENDENCY OF THE HYDROXYL ION CATALYZED REACTION.

EXPERIMENTAL

Reagents

All reagents used in this investigation were of analytical grade. Silicic acid (Mallinckrodt chromatographic grade) was used for packing the columns. All buffers were prepared with water distilled from glass apparatus, and the pH was measured on a Beckman Model G pH meter.

Procedure used for Determination of pH and Temperature Effect

Approximately 2 gms. of chloramphenicol was accurately weighed into a one-liter volumetric flask. About 900 ml. of redistilled water was added and the flask was shaken until the contents dissolved. This flask was then placed in a constant temperature bath at 60.0°C., and the solution was allowed to equilibrate thermally. Then sufficient water was added to bring the solution to the mark.

Buffer solutions of sodium carbonate- sodium bicarbonate of a normality 5 times that desired for the reaction study were prepared and likewise placed

in the constant temperature bath. 20 ml. of the buffer was pipetted into a 100 ml. volumetric flask placed in the bath, and the flask was brought to the mark with the chloramphenicol solution. At periodic intervals, 5 ml. samples were pipetted from the flask into 8 dram vials into which had been previously pipetted 5 ml. of nitric acid of sufficient strength to neutralize the base in the buffer.

These samples were retained in a refrigerator until such time as they could be chromatogrammed for the recovery of chloramphenicol. The chromatographic procedure used has been described in a previous paper (22).

The above procedures were repeated, using piperidine-piperidine hydrochloride buffers, instead of carbonate buffers. These were likewise run at 60.0°C.

Again, the same procedure was followed, but instead of buffered systems, solutions of barium hydroxide of differing normalities were used. In addition to 60.0°C., the runs were also made at 40.5°C. and at 25.0°C.

Procedure used for Determination of Chloride Ions

At the time of withdrawing 5 ml. samples from the carbonate buffer systems, an additional 5 ml. was also withdrawn and titrated amperometrically, as described in a previous paper (17).

SUMMARY

The over-all disappearance rate of chloramphenicol may be determined either by following the rate of disappearance of chloramphenicol itself or by following the rate of appearance of chloride ions. Equations for such a relationship were derived mathematically and correlated well with experimental results.

The rate of chloramphenicol degradation is directly dependent on the hydroxyl ion concentration and is reasonably first order within the ranges studied.

There is a definite buffer catalysis dependent on the pH, but its effect is greatly diminished at higher pH values, until it is completely overshadowed at pH 12.

There is some evidence that an initial reaction of 1 mole of chloramphenicol occurs with 2 moles of hydroxyl ions, but no supporting evidence could be obtained.

GENERAL SUMMARY

This study is concerned with the chemistry and chemical kinetics of the reactions responsible for loss of activity of the broad-spectrum antibiotic, chloramphenicol, in aqueous solutions. Although the drug, D-(-)-threo-1-p-nitrophenyl-2-dichloroacetamido-1,3-propanediol, is the most stable of the antibiotics known up to the present, it is subject to several degradative reactions. Hydrolysis of the amide, hydrolytic cleavage of the halogens, oxidation of the alcoholic groups and reduction of the nitro group all lead to breakdown of the compound in solution. Preliminary investigations, however, have shown that the latter two processes are of very minor importance except under very severe alkaline conditions. Accordingly, hydrolytic cleavage of the amide bond and of the halogens have been the major concern of this study.

Dehalogenation reactions are especially easy to follow kinetically because of the ease with which halogen formation may be determined analytically. The findings from this phase of the study show that the over-all reaction of chloramphenicol in water to yield chloride ions is a summation of three reactions; an uncatalyzed hydrolysis yielding chloride ions;

a hydroxyl ion catalyzed hydrolysis yielding chloride ions; and a hydrolytic cleavage of the amide bond yielding dichloroacetate ions, which in turn break down very slowly to form chloride ions. The first reaction is the predominant one at pH 6 or lower. The latter two are responsible for breakdown at higher pH, the amide cleavage being almost wholly responsible at very high pH.

In contrast to the ease with which dehalogenation reactions may be followed, amide cleavage presents more of a problem. It was desirable in such a study to be able to determine fairly precisely the actual disappearance of chloramphenicol itself from the system. Since the only assay procedure available at that time for determination of chloramphenicol was an approximate microbiological method based on turbidimetry, this was not possible. This method gave results reproducible to within only 5 per cent and was not precise enough for the purpose at hand. Therefore, a new assay method of higher precision based on chromatographic isolation was developed.

The effects of temperature, concentration, pH, buffers, and neutral salts on the rate of disappearance of chloramphenicol from aqueous solutions were investigated by means of the newly developed analytical

procedure. The results obtained showed that the over-all degradation rate of chloramphenicol in aqueous media was first order with respect to the antibiotic, independent of the ionic strength of the medium, and large independent of the hydrogen ion concentration within the pH range 2-7. Above this pH, the rate was dependent on the hydroxyl ion concentration, although the reaction between chloramphenicol and hydroxyl ions seemed to be a rather complex one. There was also a definite buffer catalysis which was dependent on pH, but this effect was much less at pH 12 and above.

A relationship was derived mathematically correlating the rate of production of chloride ions with the over-all disappearance rate of chloramphenicol.

BIBLIOGRAPHY

1. Rebstock, M.C., Crooks, H.M. Jr., Controulis, J., Bartz, Q.R., J. Am. Chem. Soc., 71, 2458 (1949).

2. Ehrlich, J., Bartz, Q.R., Smith, R.M., Joslyn, A.A., Science, 106, 417 (1947).

3. Controulis, J., Rebstock, M.C., Crooks, H.M. Jr., J. Am. Chem. Soc., 71, 2463 (1949).

4. Long, L.M., Troutman, H.D., J. Am. Chem. Soc., 71, 2469 (1949).

5. Long, L.M., Troutman, H.D., Ibid., 71, 2473 (1949).

6. "Antibiotics", The Pharmaceutical Press, London, 1952.

7. Ehrlich, J., Bartz, Q.R., et al., Science 106, 417 (1947).

8. Bartz, Q.R., J. Biol. Chem., 172, 445 (1948).

9. Bolin, I., Z. anorg. allg. Chem., 143, 201 (1925).

10. Ingold, C.K., "Structure and Mechanism in Organic Chemistry", Cornell University Press, Ithaca, 1953.

11. Crocker, J.C., Lowe, F.H., J. Chem. Soc., 91, 952 (1907).

12. Peskoff, N., Meyer, J., Z. physik. Chem., 82, 129 (1913).

13. Willems, M., Bruylants, A., Bull. soc. chim. belges, 60, 191 (1951).

14. Meloche, I., Laidler, K.J., J. Am. Chem. Soc., 73, 1712 (1951).

15. Reid, E.E., Am. Chem. J., 21, 284 (1899).

16. Reid, E.E., Ibid., 24, 397 (1900).

17. Higuchi, T., Bias, C.D., J. Am. Pharm. Assoc., 42, 707 (1953).

18. Higuchi, T., Marcus, A.D., Bias, C.D., J. Am. Pharm. Assoc., 43, 129 (1954).

19. Higuchi, T., Marcus, A.D., Ibid., in press.

20. Kunze, F., Z. physik. Chem., A188, 90 (1941).

21. Trolle-Lassen, C., Arch. Pharm. Chemi, 60, 689 (1953).

22. Higuchi, T., Marcus, A.D., Bias, C.D., J. Am. Pharm. Assoc., 43, 135 (1954).

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