THE UNIVERSITY OF MANITOBA

HYDROLYSIS OF SODIUM PYROPHOSPHATE AND SODIUM

TRIPOLYPHOSPHATE IN STERILE DISTILLED

WATER AND IN SOIL

by

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ABSTRACT

Several studies were conducted to determine the influence of incubation temperature, pH or substrate concentration on the rates of hydrolysis of pyrophosphate and tripolyphosphate in sterile distilled water and in a calcareous and a noncalcareous soil.

The rates of hydrolysis of sodium pyrophosphate and sodium tripolyphosphate in sterile distilled water were found to be very slow and follow first-order reaction kinetics. The rate of hydrolysis significantly increased with increasing temperature and slightly increased with decreasing pH from 8 to 4. There was little or no hydrolysis observed at $5^{\circ}C$ in sterile distilled water.

The rates of hydrolysis of both pyrophosphate and tripolyphosphate, when added to soil, increased linearly with temperature at temperatures of 5 to $50^{\circ}C$. The noncalcareous soil hydrolyzed the condensed phosphates at a greater rate than did the calcareous soil. Tripolyphosphate hydrolyzed at a greater rate than did pyrophosphate in both sterile water and soils. Phosphorus extracted by water was found to hydrolyze at a greater rate than phosphorus extracted by 0.5 N H_2SO_4.

The rate of hydrolysis (% hydrolysis) decreased with increases in amount of applied condensed phosphates in soils. However, the rate of orthophosphate produced increased with increases in the amounts of substrate added.

The hydrolysis of pyrophosphate followed first-order reaction kinetics when 200 ppm-P pyrophosphate was added. However, the hydrolysis of these condensed phosphates in soil may be similar to that of a Michaelis-Menten type. The rate of hydrolysis of condensed phosphates in soils was much greater than that in sterile distilled water. Even at $5^{\circ}C$, relatively large amounts of the condensed phosphates added to soil hydrolyzed.

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I   INTRODUCTION

It has long been known that condensed phosphates react with water to form less condensed phosphates and ultimately orthophosphates. It is well established that the hydrolysis of pyrophosphate and tripolyphosphate is governed by first-order kinetics in aqueous solution (Giran, 1903; Abbott, 1909). The recent development of superphosphoric acid however, has created a great interest in condensed phosphates. This acid has approximately half of its phosphorus in the orthophosphate form, about 40 per cent as pyrophosphate and the remainder in more highly condensed forms (Striplin et al., 1958). The value of pyrophosphate and tripolyphosphate as an immediate source of phosphorus to the plant is at least partially dependent upon its hydrolysis to orthophosphate (Sutton and Larsen, 1964, 1966). Hydrolysis of condensed phosphates proceeds very slowly in sterile neutral solutions at room temperature. Factors which affect the rate of hydrolysis of polyphosphate in aqueous media have been listed by Van Wazer (1958). The rate of polyphosphate hydrolysis increases with increasing temperature, hydrogen ion concentration, enzymatic activity, colloidal gels and ionic environment. There is very little information available on the effects of the above factors on rates of hydrolysis of pyrophosphosphate and tripolyphosphate in soil systems.

Since condensed phosphates are presently being incorporated into some phosphatic fertilizers used in Manitoba and these condensed phosphates have to undergo hydrolysis prior to utilization by the plant, studies were initiated to examine the behavior of pyrophosphate and tripolyphosphate in soil and aqueous solutions.

The objectives of this study were as follows:

(1) to determine the effect of pH and temperature on the

rates of hydrolysis of pyrophosphate and tripolyphosphate in sterile distilled water.

(2) to study the effects of temperature and concentration on the rate of hydrolysis of pyrophosphate and tripolyphosphate in soils.

Mechanism of Hydrolysis

The mechanism of hydrolysis of the low molecular weight phosphates such as pyrophosphate and tripolyphosphate is relatively straight forward. Simultaneous scissions of the P-O-P linkages in the same molecule-ion do not occur, and the activated complex is thought to be due to a chelation of the water, hydronium ion, or hydrated metal ion (catalyst) with a

```
        o        o
        |        |
  o  -  p  -  o  -  p  -  o        group or
        |        |
        o-      -o
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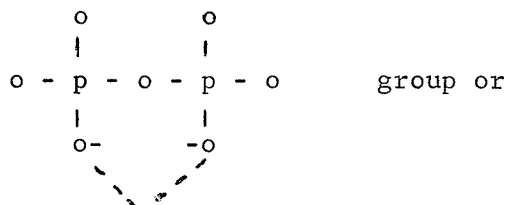
the activated complex may involve the d-orbitals of the phosphorus so that there would be five bonds proceeding from this atom in the activated complex (Van Wazer 1958). Pyrophosphate hydrolyzes to form two units of orthophosphate as follows:

$$P_2O_7^{4-} + H_2O \longrightarrow 2PO_4^{3-} + 2H^+ \quad \text{(Van Wazer 1958).}$$
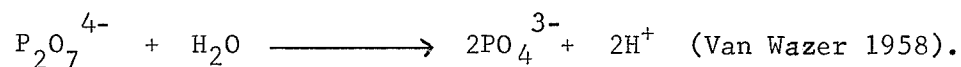
Tripolyphosphate hydrolysis includes two steps. The first step of hydrolysis of tripolyphosphate $(P_3O_{10}^{5-})$ results in the formation of a pyrophosphate $(P_2O_7^{4-})$ unit and a unit of orthophosphate (PO_4^{3-}) (Van Wazer 1958). The pyrophosphate further hydrolyzes to form two units of orthophosphate. The reactions are as follows:

$$P_3O_{10}^{5-} + H_2O \longrightarrow P_2O_7^{4-} + PO_4^{3-} + 2H^+$$
$$P_2O_7^{4-} + H_2O \longrightarrow 2PO_4^{3-} + 2H^+$$
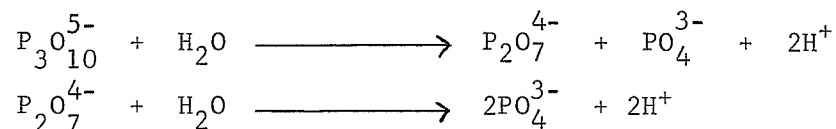
Crowther and Westman (1954) and Smith (1959) found that the hydrolysis of the tripolyphosphate ion and the pyrophosphate ion in the same dilute solution proceeded independently.

Factors Affecting Rate of Hydrolysis

(a) Aqueous systems:

The rate of hydrolysis of condensed phosphates in aqueous solution is affected by a number of factors. The major environmental factors are listed below, in what is believed to be their decreasing order of effectiveness (Van Wazer 1958).

Factor	Approximate Effect on Rate of Hydrolysis
1. Temperature	10^5-10^6 faster from freezing to boiling
2. pH	10^3-10^4 slower from strong acid to base
3. Enzymes	As much as 10^5-10^6 faster
4. Colloidal gels	As much as 10^4-10^5 faster
5. Complexing cations	Very many-fold faster in most cases
6. Concentration	Roughly proportional
7. Ionic environment in the solution	Several-fold change

In spite of the large amount of work on the study of these factors influencing the rate of hydrolysis, there are still many problems to be solved. The influence of colloidal gels and ionic environment in the solution are only roughly understood.

The effect of temperature is probably the best defined of all the major factors affecting rate of hydrolysis of condensed phosphates. Green (1950), Bell (1952), Pfanstiel and Iler (1952), Friess (1952), Brovkina (1953), Campbell and Kilpatrick (1954), and Van Wazer et al. (1955) studied the effect of temperature on the rate of hydrolysis of condensed phosphates. They found that the variation of the rate constant, k, with temperature could be denoted by the Arrhenius equation,

$$k = A\ e^{-E/RT}$$

where the temperature independent term, A is called the frequency factor, E the activation energy, and R and T the gas constant and absolute temperature, respectively. In general, these investigators have found that the activation energy for splitting P-O-P linkages in phosphate chains and rings range from 12 to 41 Kcal/mole of linkages, depending on the position of the linkage in the molecule-ion and environmental factors, such as pH and added salt concentration. An average value, 25 Kcal/mole of P-O-P linkages, which is equivalent to a doubling of the rate for about every $5^{\circ}C$ rise in temperature is often reported. Without catalysts (including H^+ and OH^-) present, the activation energy is high (Van Wazer et al., 1952, 1954), around 40 kcal/mole of P-O-P linkages, but this condition is a very rare and idealized situation. There is a drop of 12-17 kcal/mole in this value when strong acids catalyze the degradation (Van Wazer, 1958).

Morgan and Swoope (1943), Bell (1947, 1952), McGilrery and Crowther (1954), and Green (1950) have found that the hydrolytic degradation of all chain and ring phosphates was strongly catalyzed by hydrogen ions which not only lower the activation energy but also have a great effect on the frequency factor. The hydrolysis of pyrophosphate and tripolyphosphate in aqueous solution was acid catalyzed, while only tripolyphosphate was base catalyzed. Van Wazer et al. (1952) have suggested that this apparent base catalysis of tripolyphosphate hydrolysis was due to the formation of complexes with the sodium ion.

The above studies, on the effect of pH and temperature on the rates of hydrolysis of pyrophosphate and tripolyphosphate in distilled water, have been summarized by Van Wazer (1958). In general, it takes several years for 5 per cent of these condensed phosphates to hydrolyze

in distilled water maintained at pH 7.0 and room temperature.

Griffith (1959) has summarized the data of Van Wazer et al. (1955) giving the half-life of pyrophosphate and tripolyphosphate as a function of pH and temperature. He pointed out that the half-life was not a simple function of pH and temperature, when great precision is required. However, he made approximate estimates for the half-life of pyro- and tripolyphosphate at various temperatures and pHs. The half-life of pyro- and tripolyphosphate at pH 4 and 5°C was approximately 210,000 and 70,000 hours, respectively. The half-life of pyro- and tripolyphosphate at pH 4 and 20°C was approximately 21,000 and 7,000 hours, respectively. The half-life of pyro- and tripolyphosphate at pH 7 and 20°C was approximately 70,000 and 14,000 hours, respectively.

The enzymatic hydrolysis or chain and ring phosphates can be extremely rapid under certain conditions. The action of the phosphatases (Roche, 1950) is affected by small amounts of certain metal ions and hydrogen ion concentration. High temperatures and extremes in pH deactivate the phosphatases. There seems to be little doubt but that enzymes which catalyze the hydrolysis of long chain phosphatases will not affect the hydrolysis of short chain phosphates and vice versa (Ingelman and Malmgren, 1945). In general divalent cations have a pronounced effect on the enzymatic hydrolysis of P-O-P linkages. In fact, no catalysis by phosphatases will occur without the presence of divalent cations, especially magnesium (Harrow and Mazur, 1958). Pyrophosphate, tripolyphosphate, hexametaphosphate, and adenosine tripolyphosphate were incubated with pieces of Porphyra perforata in artificial sea water by Eppley (1962). The production of orthophosphate in this medium was much greater when living Porphyra tissue was present, than when boiled Porphyra

tissue was used. This activity is indicative of enzymatic catalysis. Eppley (1962) also concluded that both calcium and magnesium must be present in the medium for hydrolysis, and a Ca/Mg ratio of 0.19 yielded a maximum rate of hydrolysis.

In biological systems many phosphatases are involved in metabolic pathways and syntheses and these enzymes are quite specific. However, several reports, such as those of Forti (1961), Ito et al. (1955), and Newmark and Wenger (1960) stated that enzymes have been found which split both adenylpolyphosphates and inorganic pyrophosphate with equal facility. Unless special care is taken to insure that the system is sterile, solutions of phosphates in the parts per million concentration range undergo very rapid hydrolysis because of the action of the phosphatases liberated by microorganisms.

The effect of colloidal gels such as the hydrated oxides of iron, cobalt, nickel, aluminum, and the rare earths in accelerating the hydrolytic degradation of chain phosphates is very great (Bamann and Meisenheimer, 1958). This effect has only been studied by one group of investigators and should be more fully investigated.

Van Wazer et al. (1952, 1955) studied the hydrolysis of pyrophosphate and tripolyphosphate and showed that the usual increase in the rate at higher pH values (10-13) was absent when the system (including the added base) contained no cations except tetramethylammonium ions. Substitution of tetramethylammonium ions by sodium ions in neutral and alkaline solutions (where catalysis by hydrogen ion did not overshadow the effect) caused a several-fold increase in hydrolysis rate. This was attributed to the complexing effect of the sodium ion. Calcium ion was found to give a considerably greater increase in hydrolysis rate than did

sodium (Green, 1950; Huffman and Fleming, 1960), but it has been reported that the magnesium ion retards hydrolysis without visible formation of a precipitate (Green, 1950).

The influence of concentration of the substrate on the rate of hydrolysis of pyrophosphate and tripolyphosphate appears to be a first-order process and thus the rate is proportional to the concentration of the substrate (Crowther and Westman, 1954; Van Wazer et al., 1952, 1955). Some reports in the literature state that the rate of hydrolysis of phosphate chains increases somewhat less than in proportion to the concentration (Balareff, 1911), and, amazingly enough, trimetaphosphate hydrolysis was found to accelerate with decreasing concentration even though the process appeared to follow that of a first order reaction (Karbe, 1942). Presumably, there are several opposing effects which are included under the term "concentration".

The effect of ionic environment is difficult to separate from the other environmental factors, especially the effect of complexing cations. Van Wazer et al. (1952, 1955) used tetramethylammonium ions as the swamping electrolyte and found that the ratio of rate without swamping electrolyte to that with swamping electrolyte was larger than one in both acidic and basic solutions but the values decreased from 2.9 to 1.1 with increasing pH from 1 to 13. According to the Bronsted-Bjerrum rule, which is a theoretical treatment of the influence of ionic strength on the rates of reactions between ions (Bronsted-Bjerrum in Laidler, 1965), the rate of hydrolysis will decrease with increases in the ionic environment in both acid and basic solutions but will be catalyzed by hydrogen (hydronium)ion (Van Wazer et al., 1955).

(b) Soil Systems:

Soil is a very complicated system and not as simple as a pure water system. There are many reactions which occur which can modify the hydrolysis reaction when condensed phosphates are added to soils. All the factors which affect the rate of hydrolysis of condensed phosphates in aqueous media may be operative in a soil system but the role of each individual factor is not well understood.

The efficient utilization of pyrophosphate and tripolyphosphate as a source of phosphorus by plants depends on its rate of hydrolysis which is influenced by a number of factors. The P-O-P linkages in condensed phosphates are very moderately stable in aqueous systems at neutral pH and room temperature. Sutton and Larsen (1964) and Hashimoto et al. (1969) considered that the hydrolysis reaction of pyrophosphate in the soil is enzymatic. The effect of pH on rate of hydrolysis of condensed phosphates in soil is not clear but several possibilities exist. It may be a result of changes in the microbial population as a result of increased soil pH. It has been demonstrated by Karl-Kroupa et al. (1957) that some microorganisms are much more effective in causing the hydrolysis of pyrophosphate than others. It may also be that the pyrophosphatases produced are less effective at higher pH values. Sutton and Larson (1964) attempted to correlate the rate of hydrolysis of pyrophosphate in soil with several soil properties such as pH, per cent clay, per cent organic carbon and biological activity as determined by CO_2 evolution. They formulated an equation which could predict the half-life of hydrolysis of pyrophosphate.

$$t_{\frac{1}{2}} = \frac{1290}{C^{1.16}} \text{ X } 0.72^r$$

where $t\frac{1}{2}$ = half life in days

C = mg CO_2 evolved/day from 100 g soil

r = soil pH

They also attributed the observed pH effect to a positive correlation between pH and soil biological activity which overshadowed the direct chemical effect of pH. Gilliam and Sample (1968) found that varying the soil pH from 4.8 to 5.6 had no effect on the rate of hydrolysis of pyrophosphate. However, the rate of hydrolysis decreased at pH 6.2 and above in non-sterile soil. On the other hand, Blancher and Hossner (1969b) have recorded a slow rate of hydrolysis of tripolyphosphate below pH 6.8 and above pH 7.7. They also found that tripolyphosphate hydrolyzed to pyrophosphate and orthophosphate within 7 days but pyrophosphate persisted in the soil after 14 days. The pH of the soils varied from 5.3 to 7.8. The hydrolysis of tripolyphosphate in soil was reported to be a first order reaction (Blancher and Hossner, 1969a). Gilliam and Sample (1968) also reported that soil factors such as soil pH and biological activity were also important in determining the rate of hydrolysis of pyrophosphate in soil.

According to Van Wazer (1958), the temperature is the most important environmental factor in influencing the rate of hydrolysis of condensed phosphates. Under laboratory conditions Sutton et al. (1966) found that a temperature of 30^o — 35^oC was optimum for hydrolysis of pyrophosphate. The rate of hydrolysis was lower at soil temperatures of 7^o - 12^oC. The lower rate at the lower temperatures was apparently due to restricted biological activity. Thorup (1957) has pointed out that the rate of hydrolysis decreased as the amount of condensed phosphates which were fixed by soils increased suggesting a direct effect of chemical

factors of soils on hydrolysis rate. Sutton and Larson (1964) recorded that there was no correlation between soil colloids (clay and organic matter) and pyrophosphate hydrolysis. Hashimoto et al. (1969) reported that colloidal gels and clay minerals catalyze the hydrolysis of pyrophosphate. In very dilute solutions, kaolinite, montmorillonite and particularly goethite showed some degree of this catalytic activity, but gibbsite had no such effect. Bamann and Meisenheimer (1938) found that iron hydroxide, aluminum hydroxide and also other colloidal hydroxides catalyze the reaction. Microbial activity increased the hydrolysis greatly, and the presence of innoculated mineral suspensions further increased the hydrolysis. It was assumed that the mineral stimulated bacterial growth, and this effect was shown even with the inactive gibbsite.

Gilliam (1970) has found that roots of phosphorus-deficient plants rapidly hydrolyzed pyrophosphate to orthophosphate in nutrient solution; wheat and barley had approximately the same efficiency and both were much more effective than corn. He concluded that the increased rate of hydrolysis was a result of the presence of phosphatase on the exterior of the roots. He also found that the phosphatase activity was greater in phosphorus-deficient roots than in non-deficient roots. Savant and Racz (1971) reported that nonsterile roots had a greater phosphatase activity than sterile roots and they attributed the capacity of roots to hydrolyze pyrophosphate and tripolyphosphate more to the phosphatase activity of the rhizoplane organisms than to the phosphatase activity of roots per se.

The adsorption of pyrophosphate by soil constituents and the dissolution of minerals with the formation of new solid phases were investigated. Philen and Lehr (1967) showed that pyrophosphate and

tripolyphosphate react with soil minerals more slowly than orthophosphates and yield markedly different reaction products. Hashimoto et al. (1969) found that kaolinite and montmorillonite were particularly inert and were barely attacked by the pyrophosphate; goethite was slightly more reactive and a small amount of goethite dissolved. However gibbsite was very reactive with pyrophosphate with the formation of $Al(NH_4)_2P_2O_7OH \cdot 2H_2O$. They also found that pH and concentration of pyrophosphate were controlling factors in the precipitation of aluminum compounds in the system Al_2O_3 - NH_3 - $H_4P_2O_7$ - H_2O. Philen and Lehr (1967) also found that the structures and compositions of clay minerals markedly affected their reactions with the condensed phosphates and all clays contained small amounts of mineral impurities that contributed to the reaction products. They reported that no solid iron or aluminum compounds were formed by reaction of the condensed phosphates with the clay minerals or their impurities and the only solid reaction products were magnesium and calcium salts of the condensed phosphates. The calcium presumably was supplied by the impurities. The adsorption of the condensed phosphate ion by the soil complex is reported in the literature. Various studies showed that, in general, polyphosphates were preferentially adsorbed by soil to orthophosphate (MacIntire et al., 1937; Scott, 1958). Sutton and Larson (1964) have observed that soils with a high phosphorus-fixation capacity adsorbed greater amounts of phosphorus as pyrophosphate than as orthophosphate. They also showed, by means of Langmuir adsorption isotherms, that pyrophosphate was held less firmly than orthophosphate. On the other hand, according to Hashimoto et al. (1969), pyrophosphate is more strongly adsorbed than orthophosphate by soil. Some physico-chemical aspects of pyrophosphate adsorption were studied by Gunary (1966). He

noticed that pyrophosphate adsorption maximums were 0.6 to 9.0 times that of orthophosphate indicating that sites exist in soil which adsorb one form of phosphate but not the other. Blancher and Hossner (1969a) noticed that the relationship between phosphorus added and the solution content of phosphorus from ortho-, pyro- and tripoly-phosphate followed the Freundlich equation prediction very closely. Takefuji (1966) reported that in humus rich soils, the low fixation of tripolyphosphate resulted in the movement of the added phosphorus to lower layers.

III   MATERIALS AND METHOD

The investigations reported in this manuscript included
several individual studies. The analytical procedures employed in the
investigations are outlined below.

(A) The effects of pH and temperature on the rates of hydrolysis of

sodium pyrophosphate and sodium tripolyphosphate in sterilized

aqueous solution.

1. Sterilization of distilled water and glassware.

All glassware such as flasks and pipettes were sterilized
by autoclaving. The distilled water was sterilized by the same process.

2. Chemicals.

Reagent grade chemicals were used in all investigations.
Sodium pyrophosphate ($Na_4P_2O_7 \cdot 10H_2O$) and sodium tripolyphosphate ($Na_5P_3O_{10}$)
were sterilized by shaking with 99% ethyl alcohol for 10 minutes followed
by filtration.

3. pH of aqueous solutions.

The pH was measured using an Orion model 801 digital pH
meter equipped with glass and calomel electrodes. The electrodes were
sterilized by washing with 75% ethanol.

4. Incubation solution.

The solutions were made by dissolving sterilized sodium pyro-
phosphate and sterilized sodium tripolyphosphate in 1000 ml sterile dis-
tilled water. The concentration of the solution was 50 ppm with respect
to phosphorus (50 ppm-P). The pH of the solutions were adjusted to 4,
6, or 8 by the use of concentrated sodium hydroxide or sulfuric acid
solutions. The solutions, adjusted to various pHs were then incubated
at 5°, 20°, 35°, or $48^{\circ}C$.   Samples were taken after 3, 7, 9, 14 and 17

days of incubation for the samples incubated at 5^O, 20^O, and 35^OC and
after 1, 2, 5, 7, 9 and 11 days of incubation for the samples incubated
at 48^OC.   The amount of orthophosphate present in the solution was
measured and reported as ppm-phosphorus (ppm-P). The pH of solutions
varied slightly with time of incubation, the pHs in the acidic range in-
creased slightly and the pH in the alkaline range decreased slightly.
The pHs of the solutions were checked and adjusted every two days during
the incubation period.

     5. Measurement of phosphorus concentrations.

     (a) Orthophosphate phosphorus:

     The method of Martin and Doty as modified by Mozersky et al.
(1966) was used to determine the orthophosphate concentration of the
solutions. A Coleman Universal Model 14 Spectrophotometer was used for
all spectrophotometric measurements. All samples for analysis were main-
tained at a temperature of 20^OC to avoid error arising from the tempera-
ture effect on the rate of development and intensity of color. A suit-
able aliquot, usually 5 ml diluted with distilled water to 10 ml, was
taken and 10 ml of 1.5% ammonium molybdate in 0.5N H_2SO_4 added. The
final H_2SO_4 concentration in all solutions was 0.25N. Ten milliters of
a mixture of benzene and isobutanol (1:1 ratio) was then added and the
orthophosphate-molybdate complex extracted by shaking for thirty seconds.
The solution was allowed to stand for one minute and the organic phase
containing the orthophosphate-molybdate complex decanted. The color
intensity of the organic phase was then measured at a wave length of 370
mμ. Some hydrolysis of the pyrophosphate and tripolyphosphate occurred
during color development and extraction. Corrections were made to ac-
count for this hydrolysis. The appearance of orthophosphate in solution

was considered as a measure of the degradation of pyrophosphate and tri-polyphosphate. Although the hydrolysis of tripolyphosphate results in the formation of both pyrophosphate and orthophosphate, only the amount of orthophosphate in solution was measured.

(B) <u>Hydrolysis of polyphosphates in soil</u>.

1. Soils.

Two surface soils, varying in carbonate content, were selected. The subgroup designations and textural class names of the soils and other soil characteristics are presented in Table 1.

2. Soil pH.

The pH of a soil-water saturated paste was determined electro-metrically by the use of the glass and calomel electrodes on an Orion Model 801 digital pH meter.

3. Soil organic matter.

Soil organic matter was determined as outlined by Walkley and Black (1934). Excess potassium dichromate was used to oxidize the organic matter and the unreacted dichromate back-titrated with ferrous sulfate using barium diphenylamine sulfonate as indicator.

4. Conductivity.

The electrical conductivity of a soil-water saturated paste extract was measured using a Conductivity Bridge, Model RC16 B2.

5. Inorganic carbonate content.

A method described by Ridley (1958) was used. A one gram soil sample was digested in 10% hydrochloric acid for ten minutes. The CO_2 evolved was soaked through a drying and adsorption train, then adsorbed by Ascarite in a Nesbitt tube. The weight of CO_2 adsorbed on the Ascarite was determined and the carbonate content of the soil calculated.

Table 1

Characteristics of the soils studied

Soil Name	Newdale	Lakeland
Subgroup	Orthic Black	Gleyed Carbonated Rego Black
Textural Class	Clay Loam	Clay Loam
pH	7.5	7.9
Organic matter (%)	7.5	5.6
Conductivity (mmhos/cm)	0.8	0.5
Inorganic CO_3 (%)	1.2	18.2
$NaHCO_3$ extr. P (ppm)	15	4
Water extr. P (ppm)	3*	1*
	10**	8**
0.5 N H_2SO_4 extr. P (ppm)	155*	- ***
	195**	-

    *   Concentration of phosphorus before heating with 0.25 N H_2SO_4 at 100°C for 2 hours.

   **   Concentration of phosphorus after heating with 0.25 N H_2SO_4 at 100°C for 2 hours.

***   Not determined.

6. NaHCO$_3$ extractable phosphorus.

The extraction of NaHCO$_3$ extractable phosphorus was conducted as outlined by Olsen et al. (1954). Ten grams of soil were shaken with 100 ml of 0.5M NaHCO$_3$ extracting solution for 30 minutes. A 10 ml aliquot of the extract was transferred to a 50 ml flask. Ten milliliters of 1.5% ammonium molybdate in 0.5N H$_2$SO$_4$ was then added and the solution shaken with twenty milliliters of benzene-isobutanol (1:1) mixture for 30 seconds. The organic phase was transferred to a test tube and allowed to stand until the solution was clear. The color intensity of the organic phase was read on a Coleman Universal Model 14 Spectrophotometer at 370 mμ. By comparison of these readings with those obtained for a standard curve, the phosphorus contents of the soils were calculated.

7. Water extractable phosphorus.

A ten gram soil sample was shaken with one hundred milliliters of distilled water for 30 minutes. The solutions were then filtered and the orthophosphate concentration of the solutions measured as previously described. Total phosphorus in the water extracts was determined by the following method. Five milliliters of extract was taken and 5 ml of 0.5 N H$_2$SO$_4$ added. The solution was heated in a water bath at 100°C for two hours. Preliminary experiments showed that during two hours all the polyphosphates present in the solutions hydrolyzed to orthophosphate. The liberated orthophosphate was then determined as described above (Section 6), except that 1.5% ammonium molybdate in 0.25 N H$_2$SO$_4$ instead of 1.5% ammonium molybdate in 0.5 N H$_2$SO$_4$ was used to develop the color. The final H$_2$SO$_4$ concentration in all solutions was 0.25 N.

8. Acid extractable phosphorus.

Ten grams of soil was shaken with 100 ml of 0.5 N H$_2$SO$_4$ for

30 minutes. A 0.2 ml aliquot of extract was transferred to a 50 ml flask

and diluted to 10 mls with distilled water. Ten milliliters of 1.5%

ammonium molybdate in 0.5N H_2SO_4 was then added and the solution shaken

with twenty milliliters of benzene-isobutanol (1:1) mixture for 30

seconds. The organic phase was transferred to a test tube and allowed

to stand until the solution was clear. The color intensity of the or-

ganic phase was read on a Coleman Universal Model 14 Spectrophotometer

at 370 mu. By comparison of these readings with these obtained for a

standard curve, the phosphorus contents of the soils were calculated.

Another 0.2 ml aliquot of extract was then taken and 9.8 mls of 0.25N

H_2SO_4 added. The total phosphorus was obtained by heating this solution

in a water bath for two hours at $100^{o}C$. The liberated orthophosphate was

then determined as described above, except that 1.5% ammonium molybdate

in 0.25N H_2SO_4 instead of 0.5N H_2SO_4 was used to develop the color.

        9. Incubation for sodium pyrophosphate and sodium tripoly-

            phosphate hydrolysis in soil.

        Experiment 1, The influence of temperature on the rate of

sodium pyrophosphate and sodium tripolyphosphate hydrolysis in soil.

        Four milliliters of 500 ppm-P sodium pyrophosphate or

sodium tripolyphosphate solution were added to 10 grams of air dry soil

which was contained in a polyethylene plastic cylinder (1" dia. X $2\frac{1}{2}$").

The soil samples were incubated at 5^{o}, 20^{o}, or $35^{o}C$. After incubation

for 6, 12, 24, 36, 48, 72, or 120 hours, the phosphorus was extracted

using distilled water. The orthophosphate and total phosphorus contents

of the extracts were determined as described previously. The polyphosphate

content of the extracts was obtained by subtraction of the orthophosphate

content from the total phosphorus content. The water extractable

phosphorus content of the soils not treated with phosphorus was also determined and these values used to correct for native soil phosphorus levels.

Experiment 2. The influence of temperature on the rate of sodium pyrophosphate hydrolysis in soil.

This experiment was designed to gain information on the optimum temperature for sodium pyrophosphate hydrolysis in soil. The preparation of samples was the same as described in Experiment 1. The soil samples were incubated at 5°, 20°, 35°, 50° or 65°C.

Samples of the Lakeland soil were obtained after 6, 12, 24, or 36 hours of incubation. Samples of the Newdale soil were obtained after 6, 24, 48, 168, or 336 hours of incubation. The water extractable orthophosphate, total phosphate and polyphosphate content of the samples were determined as described for Experiment 1. Following extraction of the Newdale soil with water, the soil samples were extracted with 100 mls of 0.5N H_2SO_4 solution. The orthophosphate, total phosphate and polyphosphate contents of the acid extracts were then determined. All measurements were corrected for native soil phosphorus levels.

Experiment 3. The influence of substrate concentration on sodium pyrophosphate and sodium tripolyphosphate hydrolysis in soil.

Four milliliters of 500 ppm, 1000 ppm, 2000 ppm or 4000 ppm-P sodium pyrophosphate or sodium tripolyphosphate solution were added to 10 grams of air dry Lakeland or Newdale soil contained in polyethylene plastic cylinders. The soil samples were incubated at 20°C and after incubation for 6, 12, 24, 36, 48, 72, or 120 hours the water extractable orthophosphate, total phosphorus, and polyphosphate contents of the samples determined.

IV   RESULTS AND DISCUSSION

(A) Hydrolysis of Sodium Pyrophosphate and Sodium Tripolyphosphate in
Sterile Distilled Water.

The amount of orthophosphate hydrolyzed from sodium pyrophos-
phate and sodium tripolyphosphate in sterile distilled water maintained
at pHs of 4, 6, or 8 and at temperatures of 5, 20, 35, or 48°C are shown
in Tables 2 and 3. There was no hydrolysis of either pyrophosphate or
tripolyphosphate at 5°C (Tables 2 and 3). The amount of orthophosphate
liberated from the phosphates increased with time. The amount of ortho-
phosphate liberated from the phosphates increased with increasing tempera-
ture and decreasing pH. In general, the amount of orthophosphate libera-
ted from sodium pyrophosphate was higher than that from sodium tripoly-
phosphate at the same pH, temperature and time of incubation. The
greatest amount of orthophosphate hydrolyzed was 19.20 ppm for sodium
pyrophosphate and 13.10 ppm for sodium tripolyphosphate.

Several investigators have shown that pyrophosphate, under
sterile conditions, follows first-order reaction kinetics (Clesceri and
Lee, 1965; Gilliam and Sample, 1968). Thus when

$$P_2O_7^{4-} + H_2O \xrightarrow{k_2} 2PO_4^{3-} + 2H^+$$
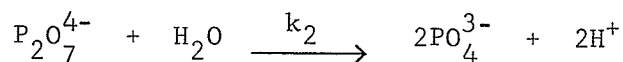
the rate of reaction at any time, t, is given by the equation:

$$- \frac{dP_2}{dt} = k_2P_2 \qquad (1)$$

where P_2 is the concentration of pyrophosphate in moles/l at any time,

k_2 the rate constant for the hydrolysis of pyrophosphate,

and t the time at which a value for P_2 was recorded.

According to the law of conservation of mass, the concentration (moles/l)
of unhydrolyzed pyrophosphate should be equal to the difference between

Table 2

Effect of pH and temperature on the amount of orthophosphate hydrolyzed
from a 50 ppm-P sodium pyrophosphate aqueous solution

Incubation temperature $^{\circ}C$	Solution pH	Concentration of orthophosphate (ppm-P) at various times of incubation				
		3 days	7 days	9 days	14 days	17 days
5	4	0	0.10	0	0	0
	6	0	0	0.20	0	0
	8	0	0.10	0	0.10	0
20	4	0.50	1.10	1.40	2.10	2.70
	6	0.30	1.00	1.20	1.70	2.20
	8	0.40	0.90	1.10	1.60	2.00
35	4	2.20	5.60	7.20	10.10	12.30
	6	1.30	4.20	5.90	8.00	9.70
	8	1.40	3.20	4.40	6.20	8.00
48		1 day	2 days	5 days	7 days	9 days	11 days
	4	1.90	3.40	10.00	12.00	16.00	19.20
	6	1.50	3.85	8.87	11.50	15.00	18.10
	8	1.00	2.70	8.20	11.50	13.88	16.50

Table 3

Effect of pH and temperature on the amount of orthophosphate hydrolyzed
from a 50 ppm-P sodium tripolyphosphate aqueous solution

Incubation temperature °C	Solution pH	Concentration of orthophosphate (ppm-P) at various times of incubation					
		3 days	7 days	9 days	14 days	17 days	
5	4	0	0.10	0.10	0	0	
	6	0	0.15	0	0.15	0	
	8	0	0.10	0.10	0	0.10	
20	4	0.55	1.34	1.65	2.34	3.00	
	6	0.40	0.95	1.10	1.82	2.34	
	8	0.35	0.90	1.00	1.64	1.85	
35	4	2.00	4.00	5.00	7.10	8.10	
	6	1.60	3.60	4.40	6.35	7.36	
	8	1.20	2.80	3.40	5.50	6.57	
48		1 day	2 days	5 days	7 days	9 days	11 days
	4	1.20	3.70	8.00	10.00	11.60	13.10
	6	1.20	2.50	6.65	8.50	10.00	11.50
	8	1.00	1.50	5.40	7.05	8.45	9.65

one half of the orthophosphate concentration (moles/l) and the initial

pyrophosphate concentration (moles/l) as shown below:

$$P_2 = P_2^O - \tfrac{1}{2}P_1 \qquad (2)$$

where, P_1 is the concentration of orthophosphate in moles/l

at any time,

and P_2^O the initial pyrophosphate concentration in moles/l.

Integration of equation (1) and substitution of equation (2) for P_2 into

equation (1) gives the following equations:

$$\frac{P_2^O - \tfrac{1}{2}P_1}{P_2^O} = e^{-k_2 t} \quad (3) \qquad OR \qquad \ln\frac{P_2^O - \tfrac{1}{2}P_1}{P_2^O} = k_2 t \quad (4)$$

If the concentration units are changed from moles/l to ppm-P, equation

(4) is modified as follows:

$$\ln\frac{P_2^{O*} - P_1^{*}}{P_2^{O*}} = -k_2 t \qquad (5)$$

where, P_2^{O*} and P_1^{*} are the concentrations of pyrophosphate

and orthophosphate in ppm-P, respectively.

The linearity of the plot of the natural logarithm of $\dfrac{P_2^{O*} - P_1^{*}}{P_2^{O*}}$

versus time in Figure 1 shows that the breakdown of pyrophosphate followed

first-order kinetics. The rate constants for this reaction at various

pHs and temperatures were calculated from the slopes of the lines shown

in Figure 1 and are listed in Table 4. The rate constants increased with

increasing temperature and decreasing pH. In this experiment the greatest

rate constant was found to be 3.19×10^{-5} min.$^{-1}$ and the lowest rate con-

stant was 1.73×10^{-6} min.$^{-1}$ for sodium pyrophosphate. Very little or

no pyrophosphate hydrolyzed at 5^OC and thus estimates of the rate con-

stants at 5^OC were not attempted.

The rate of hydrolysis of tripolyphosphate in sterile distilled
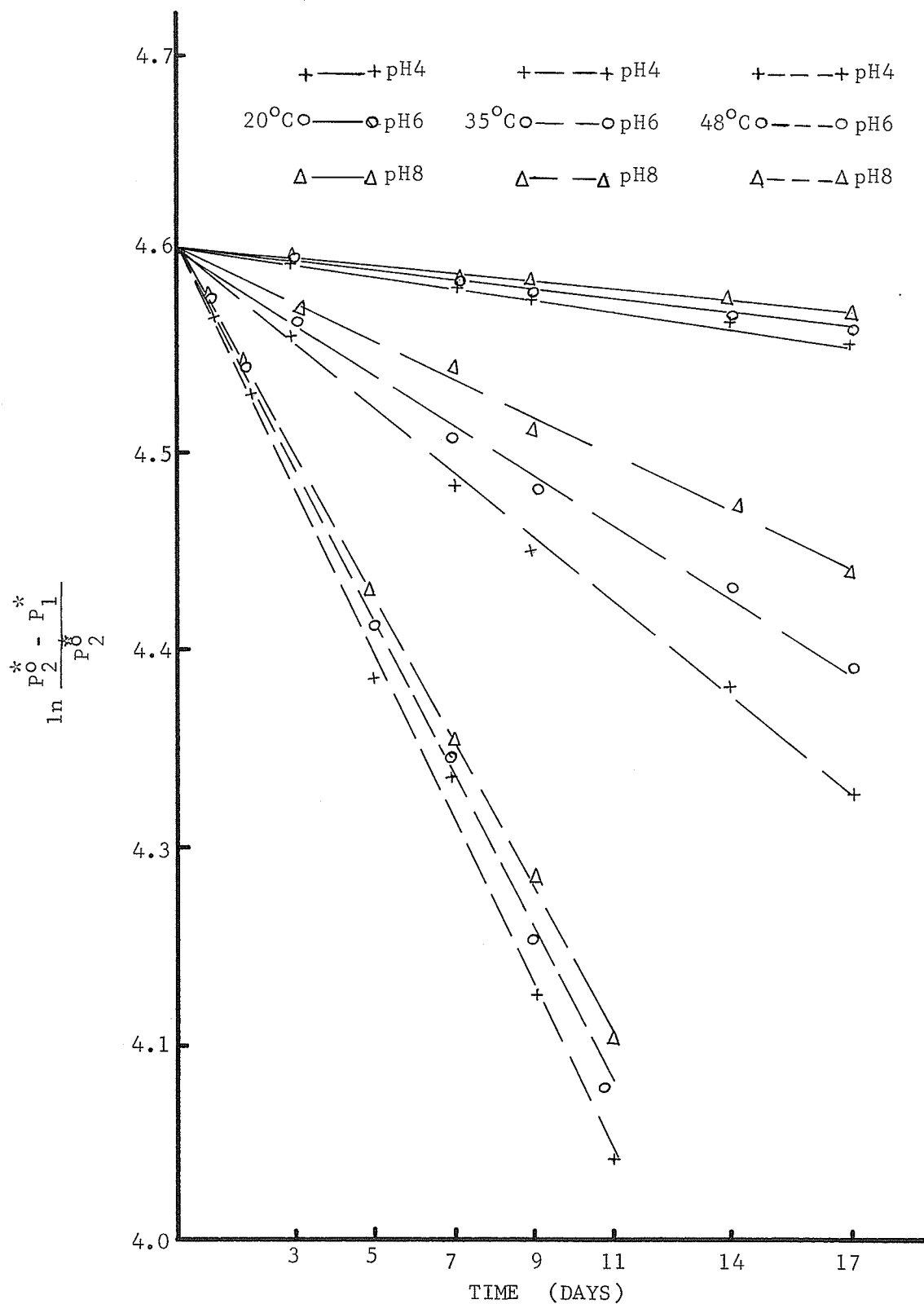
Figure 1. Plot of $\ln \dfrac{P_2^{\overset{*}{O}} - P_1^*}{P_2^{\overset{*}{O}}}$ versus time at various pHs and temperatures

for sodium pyrophosphate hydrolysis.

Table 4

Effect of pH and temperature on the first-order reaction rate
constant for sodium pyrophosphate hydrolysis

Temperature $^{\circ}$C	pH	rate constant (min^{-1})
20	4	2.26×10^{-6}
	6	1.90×10^{-6}
	8	1.73×10^{-6}
35	4	1.16×10^{-5}
	6	9.05×10^{-6}
	8	7.16×10^{-6}
48	4	3.19×10^{-5}
	6	2.83×10^{-5}
	8	2.54×10^{-5}

water was evaluated by determining orthophosphate at various incubation
times. The hydrolysis of tripolyphosphate includes two independent steps
(Smith, 1959) as shown below:

$$P_3O_{10}^{5-} + H_2O \xrightarrow{k_3} P_2O_7^{4-} + PO_4^{3-} + 2H^+$$

$$P_2O_7^{4-} + H_2O \xrightarrow{k_2} 2PO_4^{3-} + 2H^+$$
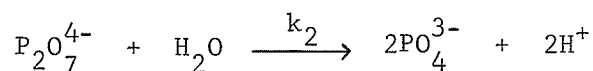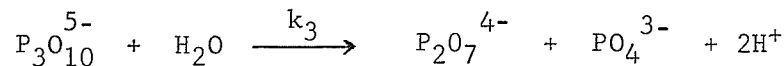
where k_3 and k_2 are the first-order rate constants for the hydrolysis
of tripolyphosphate and pyrophosphate, respectively. The simultaneous
differential equations for hydrolysis of tripolyphosphate are:

$$-\frac{dP_3}{dt} = k_3P_3 \qquad (6)$$

$$\frac{dP_2}{dt} = k_3P_3 - k_2P_2 \qquad (7)$$

$$\frac{dP_1}{dt} = 2k_2P_2 + k_3P_3 \qquad (8)$$

The solutions for equations (5), (6), and (7) are as follows:

$$P_3 = P_3^o e^{-k_3t} \qquad (9)$$

$$P_2 = \frac{k_3P_3}{(k_2-k_3)}\left[e^{-k_3t} - e^{-k_2t}\right] \qquad (10)$$

$$P_1 = \frac{2k_3k_2P_3^o}{k_2-k_3}\left\{\left(\frac{1}{k_3} - \frac{1}{k_2}\right) - \left(\frac{1}{k_3}e^{-k_3t} - \frac{1}{k_2}e^{-k_2t}\right)\right\} + P_3^o (1-e^{-k_3t}) \qquad (11)$$

Equation (11) can be rearranged as follows:

$$\frac{P_3^o - P_1}{P_3^o} = e^{-k_3t} - \frac{2k_3k_2}{k_2-k_3}\left\{\left(\frac{1}{k_3}-\frac{1}{k_2}\right) - \left(\frac{1}{k_3}e^{-k_3t} - \frac{1}{k_2}e^{-k_2t}\right)\right\} \qquad (12)$$

where k_3 and k_2 are as defined above,

P_1 the orthophosphate concentration in moles/l at any time,

P_2 and P_3 the concentrations of pyrophosphate and tripoly-
phosphate in moles/l at any time, respectively,

P_3^O the initial tripolyphosphate concentration in moles/l,

and t the time in hours at which a value for P_1 was recorded.

If the concentration units are changed from moles/l to ppm-P, equation

(12) is modified as follows:

$$\frac{\dfrac{P_3^{O*}}{3} - P_1^{*}}{\dfrac{P_3^{O*}}{3}} = e^{-k_3 t} - \frac{2k_3 k_2}{k_2 - k_3} \left\{ (\frac{1}{k_3} - \frac{1}{k_2}) - (\frac{1}{k_3} e^{-k_3 t} - \frac{1}{k_2} e^{-k_2 t}) \right\} \quad (13)$$

where P_3^{O*} and P_1^{*} are the initial tripolyphosphate and orthophos-

phate concentrations in ppm-P at anytime, respectively.

During the initial stages of hydrolysis, due to the presence of only

small amount of pyrophosphate, the term

$$\frac{2 k_3 k_2}{k_2 - k_3} \left\{ (\frac{1}{k_3} - \frac{1}{k_2}) - (\frac{1}{k_3} e^{-k_3 t} - \frac{1}{k_2} e^{-k_2 t}) \right\}$$

is much smaller than $e^{-k_3 t}$ in equation (13) and the following approximative

equation can be used:

$$\frac{P_3^{O*}/3 - P_1^{*}}{P_3^{O*}/3} = e^{-k_3 t} \quad (14)$$

$$\text{or} \quad \ln \frac{\dfrac{P_3^{O*}}{3} - P_1^{*}}{\dfrac{P_3^{O*}}{3}} = -k_3 t \quad (15)$$

A plot of $\ln(\dfrac{\dfrac{P_3^{O*}}{3} - P_1^{*}}{\dfrac{P_3^{O*}}{3}})$ versus time should yield a straight line with a

slope of $-k_3$ at an early stage of hydrolysis. From the slopes of straight

lines in Figure 2 in which $\ln \dfrac{P_3^{O*}/3 - P_1^{*}}{P_3^{O*}/3}$ was plotted versus time, the first
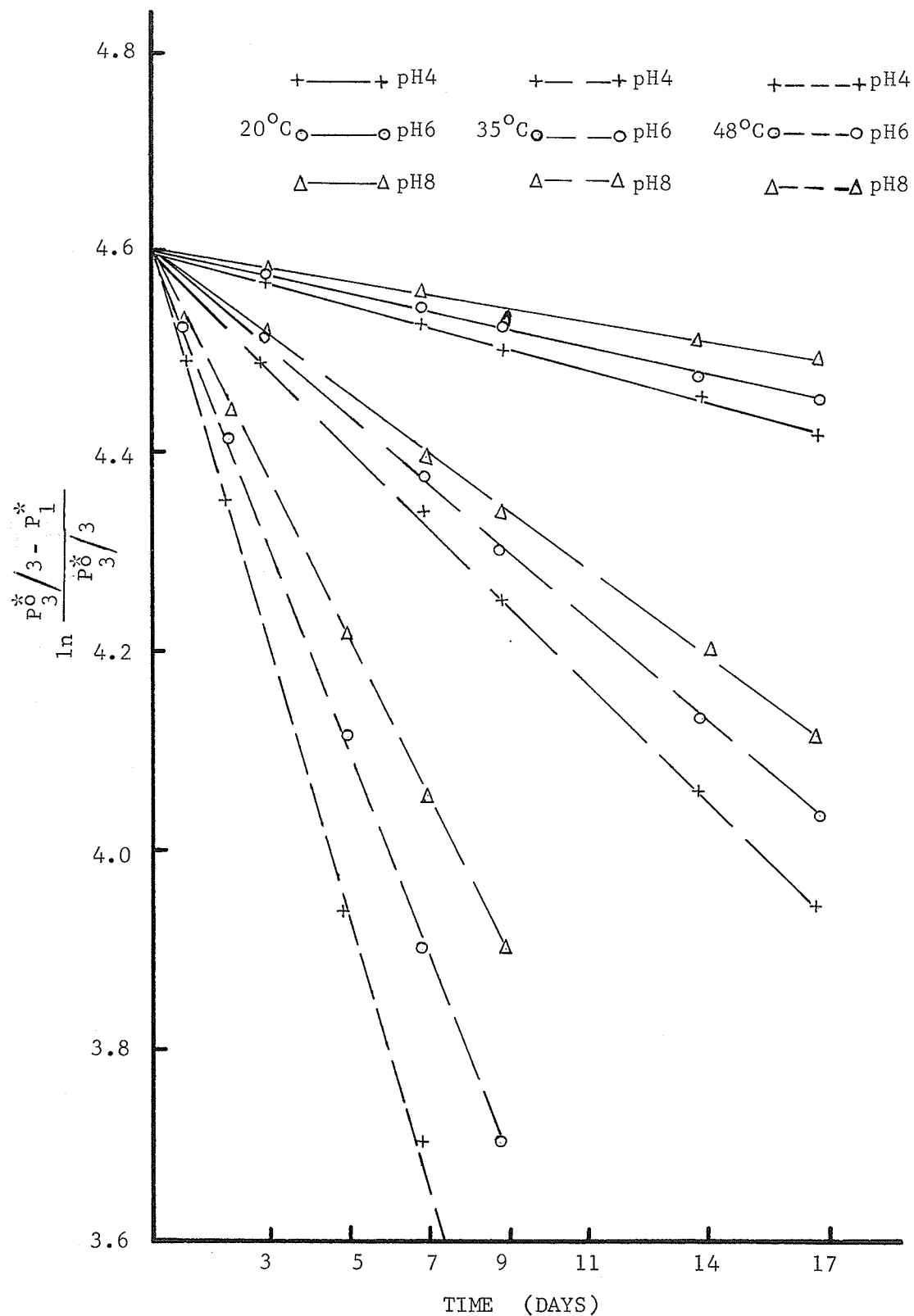
Figure 2. Plot of $\ln \dfrac{P_3^{*o}/3 - P_1^*}{P_3^{*o}/3}$ versus time at various pHs and temperatures

for sodium tripolyphosphate hydrolysis.

step of hydrolysis characterized by k_3 was calculated. After 9 days of incubation at 48°C, the concentration of pyrophosphate produced was high and the lines started to bend. The values for the reaction rate constants are listed in Table 5. The rate constants or the slopes of the lines increased with increasing temperature and decreasing pH. The greatest rate constant was found to be 8.72 X 10^{-5}/min. and the lowest was 4.87 X 10^{-6}/min. for sodium tripolyphosphate.

The rate of hydrolysis was very slow for both the pyrophosphate and tripolyphosphate solution over the temperature and pH range studied. The rate of hydrolysis increased with increases in temperature at all pHs. The influence of temperature on the rate of hydrolysis can be predicted by the equation,

$$\frac{d \ln k}{dT} = \frac{Ea}{RT^2} \qquad (16)$$

$$\text{or} \quad \ln k = -\frac{Ea}{RT} + C \qquad (17)$$

where k   is the rate constant of reaction,

   T   the absolute temperature,

   R   the gas constant,

   Ea   the activation energy for the reaction,

and   C   an integration constant.

From equation (17), it follows that a plot of the natural logarithm of the rate constant versus the reciprocal of the absolute temperature should be a straight line with a slope of -Ea/R. The natural logarithm of the rate constants for hydrolysis of pyrophosphate and tripolyphosphates versus 1/T are plotted in Figures 3 and 4. The straight lines, obtained in Figures 3 and 4, showed that the data obtained followed the Arrhenius equation very well. The pH did not affect the slopes of these straight lines.

Table 5

Effect of pH and temperature on the first-order reaction rate
constant for sodium tripolyphosphate hydrolysis

Temperature $^\circ$C	pH	rate constant (min^{-1})
20	4	7.83×10^{-6}
	6	6.10×10^{-6}
	8	4.87×10^{-6}
35	4	2.74×10^{-5}
	6	2.38×10^{-5}
	8	2.05×10^{-5}
48	4	8.72×10^{-5}
	6	7.08×10^{-5}
	8	5.46×10^{-5}
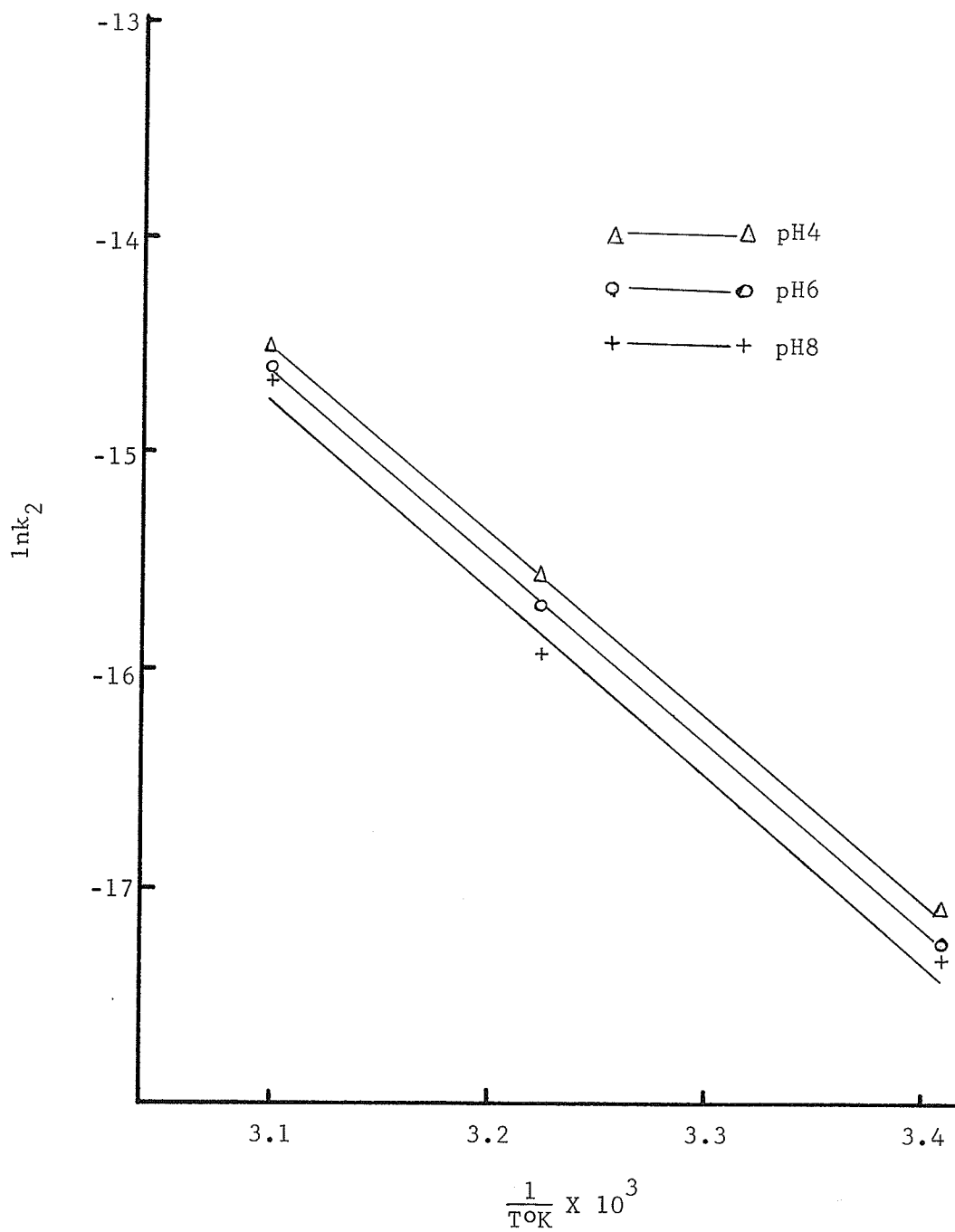
Figure 3. Plot of natural logarithm of rate constant for sodium pyrophos-
phate hydrolysis ($\ln k_2$) versus reciprocal of temperature ($1/T$)
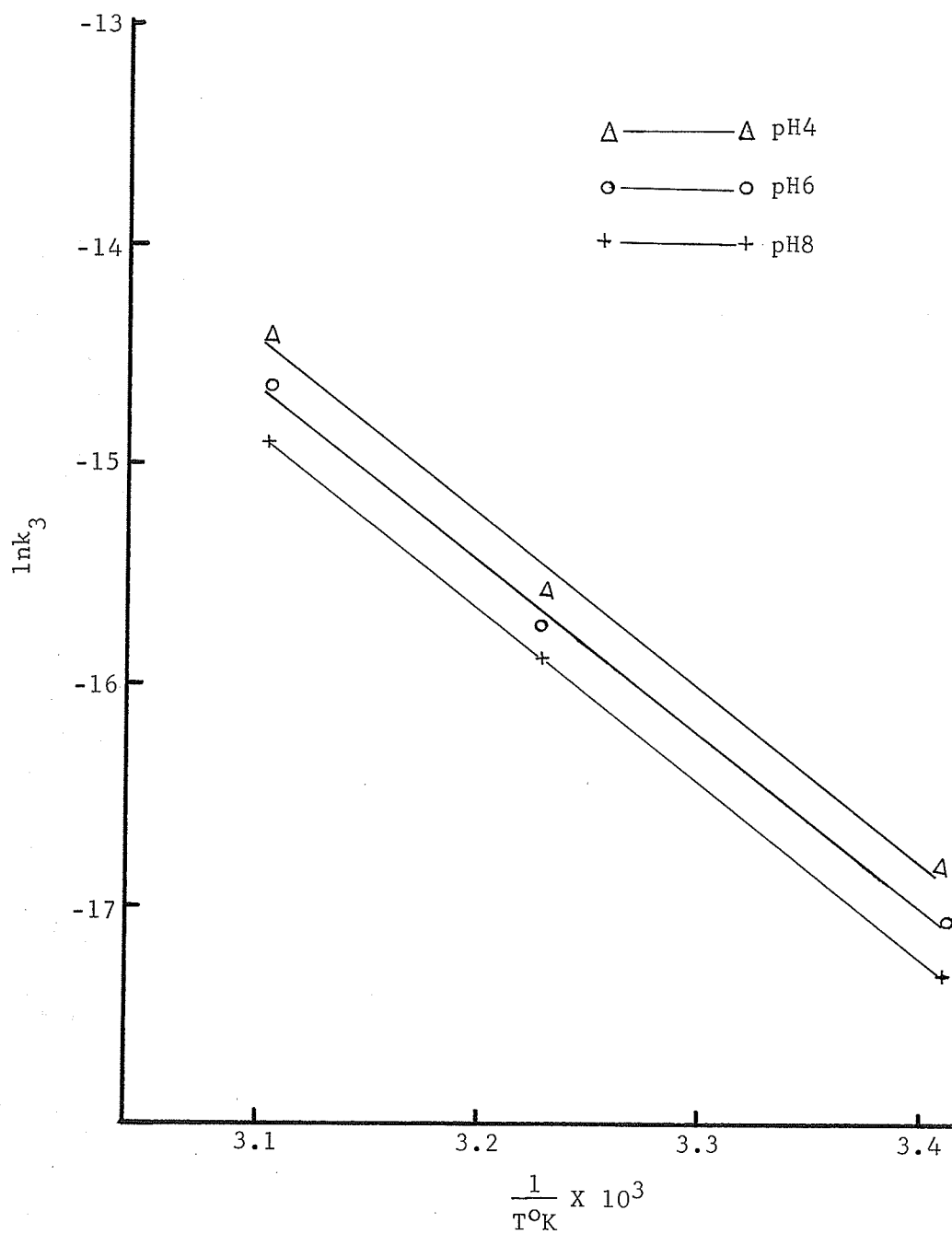
Figure 4. Plot of natural logarithm of rate constant for sodium tripoly-
phosphate hydrolysis (lnk_3) versus reciprocal of temperature (1/T)

The activation energies for the hydrolysis of pyrophosphates and tripolyphosphates were calculated from the slopes of the lines and are listed in Table 6. The average value for the activation energy for the splitting of the P-O-P linkages in pyrophosphate was 18 Kcal/mole and that for tripolyphosphate was 16.2 Kcal/mole over the temperature range studied. Data summarized by Van Wazer (1958) showed that the activation energy for the splitting of the P-O-P linkages varied from 12 to 40 Kcal/mole. The values in Table 6 show that the temperature and pH of incubation did not significantly affect the activation energy for the hydrolysis of polyphosphates in aqueous solution.

In solutions of pyrophosphate and tripolyphosphate, the effect of solution pH on the rate of hydrolysis was less important than that of temperature. At 5°C, no or little hydrolysis occurred during the sampling period regardless of the pH of the solution. The rate of hydrolysis however, decreased as the pH of the solution increased at temperatures of 20, 35 and 48°C. The hydrolytic degradation of pyrophosphate and tripolyphosphate is generally catalyzed by hydrogen ions. This is illustrated by Figures 5 and 6 in which the natural logarithm of the rate constant is plotted versus pH. The increase in rate of hydrolysis of sodium tripolyphosphate in basic solution reported by Crowther and Westman (1954) was not observed over the pH range studied. They found that the rate of hydrolysis of sodium tripolyphosphate was catalyzed by base in the pH range of 10-13.

The effect of pH and temperature on the hydrolysis of sodium pyrophosphate and tripolyphosphate were similar. The rate of hydrolysis increased with increasing temperature and decreased with increasing pH for both sodium pyrophosphate and sodium tripolyphosphate. Although

Table 6

Activation energies for the hydrolysis of sodium pyrophosphate
and sodium tripolyphosphate

Phosphate source	pH	Activation energy (Kcal/mole)	Average value (Kcal/mole)
Sodium pyrophosphate	4	17.8	
	6	18.1	18.0
	8	17.9	
Sodium tripolyphosphate	4	16.1	
	6	16.4	16.2
	8	16.1	
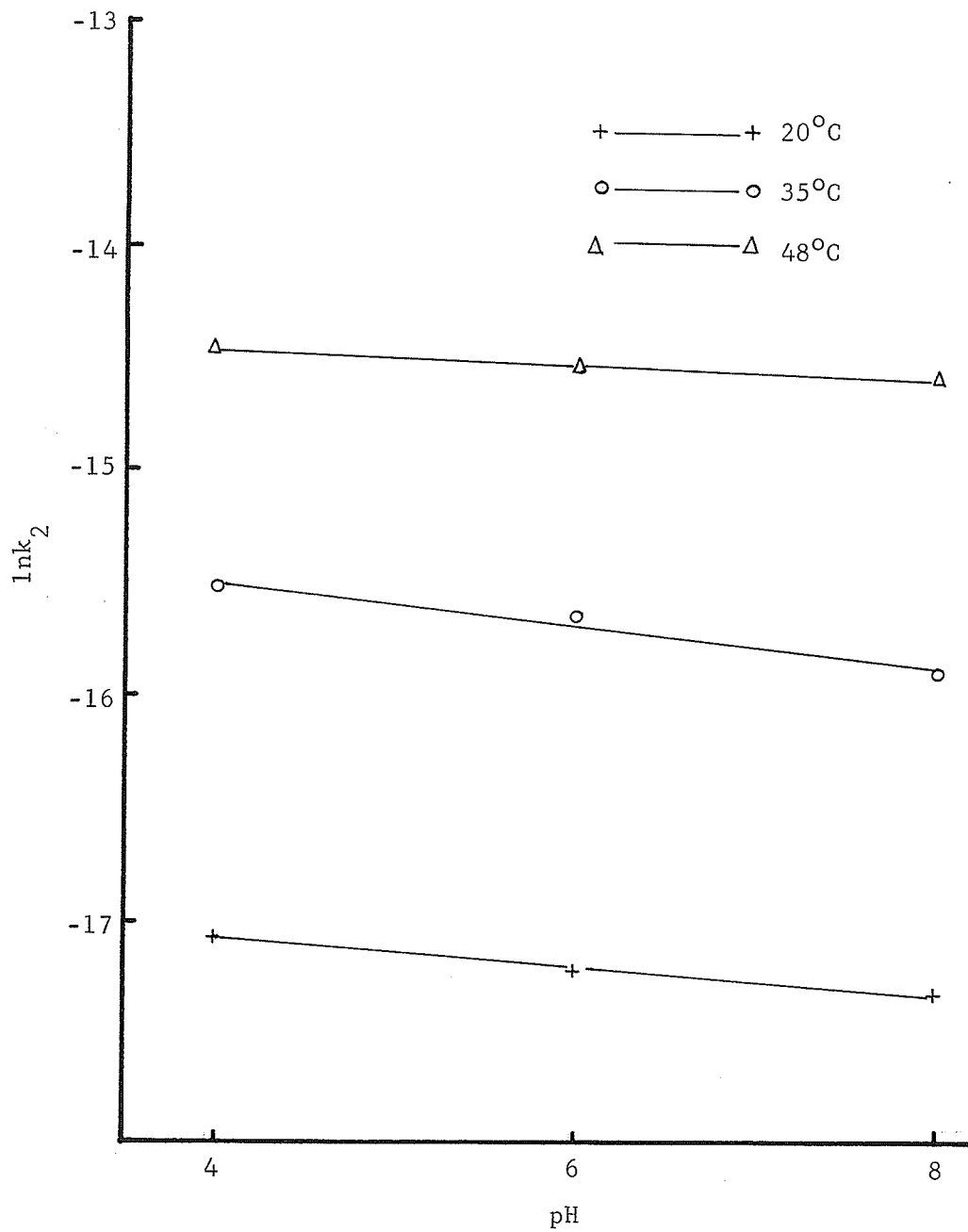
Figure 5. Plot of natural logarithm of rate constant of sodium pyrophosphate hydrolysis (lnk_2) versus solution pH.
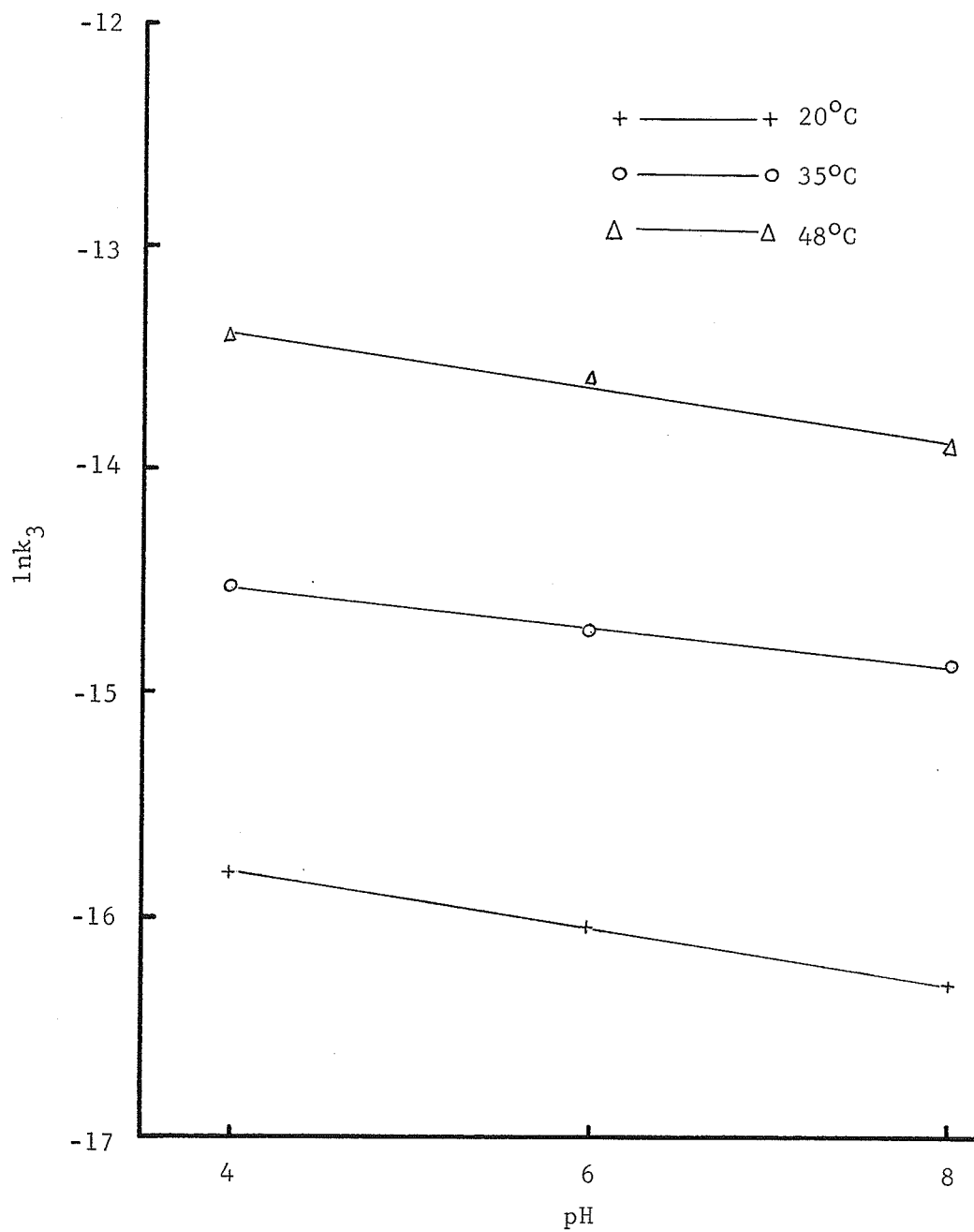
Figure 6. Plot of natural logarithm of rate constant of sodium tripoly-
phosphate hydrolysis (lnk_3) versus solution pH.

the amount of orthophosphate liberated from sodium pyrophosphate was greater than that from sodium tripolyphosphate at the same pH, temperature and time, the rate constant for the disappearance of sodium pyrophosphate from solution was smaller than that of sodium tripolyphosphate in sterile distilled water.

(B) Hydrolysis of Polyphosphate in Soil.

Experiment 1. The influence of temperature on the rates of sodium pyrophosphate and sodium tripolyphosphate hydrolysis in soil.

It is necessary to recover a high percentage of the added pyrophosphate or tripolyphosphate in order to obtain a true estimate of their degree of hydrolysis. It has been shown that acid extractants usually give the highest recovery of added pyrophosphate or tripolyphosphate (Hossner and Melton, 1970). However, in this experiment water was used as an extractant which recovered only 10-40% of the added phosphorus (Tables 7 to 10). Water was used as an extractant as water extracts the phosphorus which was not fixed by the soil or only weakly fixed and thus probably recovers a fraction readily available to plants.

The amounts of orthophosphate, polyphosphates (pyrophosphate or tripolyphosphate) and total phosphorus extracted by water from the soils incubated at 5, 20 or 35°C are shown in Tables 7 to 10. The recovery of added phosphorus (total phosphorus) from the Lakeland soil was lower than that from the Newdale soil. This may be due to the high calcium carbonate content of the Lakeland soil. The calcium carbonate in the Lakeland soil may have reacted with the pyrophosphate and tripolyphosphate to form less soluble compounds than was formed in the Newdale soil. In each soil, the recovery of tripolyphosphate was

Table 7

Effect of incubation temperature and time on the amounts of orthophosphate, pyrophosphate
and total phosphorus extracted by water from the Lakeland soil treated
with 200 ppm-P pyrophosphate

Incubation temperature (°C)		Phosphorus extracted (ppm-P) at various times of incubation						
		6 hours	12 hours	24 hours	36 hours	48 hours	72 hours	120 hours
5	total phosphorus	43.0	44.7	44.0	41.0	38.0	47.0	46.0
	orthophosphate	5.2	9.7	16.2	15.2	19.4	21.0	20.0
	pyrophosphate	37.8	35.0	27.8	26.0	18.6	26.0	26.0
20	total phosphorus	35.9	35.4	39.6	43.8	46.0	46.0	48.0
	orthophosphate	7.9	14.4	20.0	26.3	29.2	30.0	30.4
	pyrophosphate	28.0	21.0	19.6	17.5	16.8	16.0	17.6
35	total phosphorus	33.0	32.1	39.4	35.6	37.2	37.0	26.0
	orthophosphate	20.0	21.3	31.1	28.8	32.0	33.0	23.4
	pyrophosphate	13.0	10.8	8.3	6.8	5.2	4.0	2.6

Table 8

Effect of incubation temperature and time on the amounts of orthophosphate, pyrophosphate and total phosphorus extracted by water from the Newdale soil treated with 200 ppm-P pyrophosphate

Incubation temperature (°C)		Phosphorus extracted (ppm-P) at various times of incubation						
		6 hours	12 hours	24 hours	36 hours	48 hours	72 hours	120 hours
5	total phosphorus	57.2	61.0	59.6	62.6	62.5	70.0	66.4
	orthophosphate	17.2	27.0	30.4	35.1	36.5	43.0	43.0
	pyrophosphate	40.0	34.0	29.2	27.5	26.0	27.0	23.4
20	total phosphorus	57.2	56.4	58.7	61.0	65.5	75.0	79.8
	orthophosphate	27.0	33.0	36.7	40.0	44.9	54.8	60.8
	pyrophosphate	30.2	23.4	22.0	21.0	20.6	20.2	19.0
35	total phosphorus	63.0	58.4	73.0	73.1	77.5	78.0	64.6
	orthophosphate	37.0	41.8	58.4	59.8	64.5	65.9	54.0
	pyrophosphate	26.0	16.6	14.6	13.3	13.0	12.1	10.6

Table 9

Effect of incubation temperature and time on the amounts of orthophosphate, polyphosphate
and total phosphorus extracted by water from the Lakeland soil treated
with 200 ppm-P tripolyphosphate

Incubation temperature (°C)		Phosphorus extracted (ppm-P) at various times of incubation						
		6 hours	12 hours	24 hours	36 hours	48 hours	72 hours	120 hours
5	total phosphorus	55.4	53.2	47.0	41.0	38.0	39.0	45.0
	orthophosphate	12.2	16.2	21.0	22.0	23.0	25.0	30.0
	polyphosphate	43.2	37.0	26.0	19.0	15.0	14.0	15.0
20	total phosphorus	41.2	37.0	39.0	26.0	28.6	34.1	36.2
	orthophosphate	11.0	16.8	20.0	16.6	19.5	24.9	27.2
	polyphosphate	30.2	20.2	19.0	9.4	9.1	9.2	9.2
35	total phosphorus	30.4	25.0	29.2	32.0	33.0	36.4	39.0
	orthophosphate	18.4	16.2	26.0	29.2	31.0	34.4	37.2
	polyphosphate	12.0	8.8	3.2	2.8	2.0	2.0	1.6

Table 10

Effect of incubation temperature and time on the amounts of orthophosphate, polyphosphate
and total phosphorus extracted by water from the Newdale soil treated with
200 ppm-P tripolyphosphate

Incubation temperature ($^{\circ}$C)		Phosphorus extracted (ppm-P) at various times of incubation						
		6 hours	12 hours	24 hours	36 hours	48 hours	72 hours	120 hours
5	total phosphorus	75.0	77.0	75.0	64.6	66.7	69.0	67.4
	orthophosphate	25.0	32.6	35.6	39.0	46.7	51.4	50.4
	polyphosphate	50.0	44.4	39.4	25.6	20.0	17.6	17.0
20	total phosphorus	69.0	66.0	64.6	65.6	62.0	60.5	61.0
	orthophosphate	39.0	42.9	47.0	49.0	48.0	47.5	49.0
	polyphosphate	30.0	23.1	17.6	16.6	14.0	13.0	13.0
35	total phosphorus	62.0	54.0	56.4	50.3	46.8	50.0	53.2
	orthophosphate	35.0	47.0	50.6	46.0	43.5	47.0	50.6
	polyphosphate	27.0	7.0	5.8	4.3	3.3	3.0	2.6

higher than that of pyrophosphate. This can be attributed to the lower
sorption capacity of soil for tripolyphosphate than for pyrophosphate
(Blanchar and Hossner, 1969a). The amounts of total phosphorus recovered
from the soils varied with time of incubation. In some instances the
amount of phosphorus recovered increased with time of incubation. This
may be due to a greater attraction of the soil for polyphosphate than for
orthophosphate and upon hydrolysis of the polyphosphate to orthophosphate
greater amounts of phosphorus were recovered. The amounts of orthophos-
phate extracted from the soils usually increased with time of incubation
and incubation temperature. The amount of pyrophosphate and tripolyphos-
phate extracted from the soils decreased with time of incubation and
incubation temperature.

The rate of hydrolysis of pyrophosphate or tripolyphosphate,
expressed as a percentage of the phosphates extracted, (amount of ortho-
phosphate extracted/amount of total phosphorus extracted X 100), in the
Lakeland and Newdale soils are shown in Figures 7 to 10. The rate of
hydrolysis of polyphosphate in both soils was extremely rapid and much
greater than in sterile distilled water. Even at $5^{\circ}C$ and pH around
neutral, there was an appreciable amount of polyphosphate hydrolyzed.
This is probably due to the high enzymatic activity of soils. The hy-
drolysis of both phosphates followed a similar pattern. The phosphates
hydrolyzed extremely rapidly during the first two to three days of in-
cubation. This rapid rate of hydrolysis was followed by a very slow rate
of hydrolysis which continued for several days. It is interesting to
note that even at $35^{\circ}C$, not all the added phosphate was hydrolyzed at the
end of the incubation period.

Both the rate and extent of hydrolysis of the phosphates
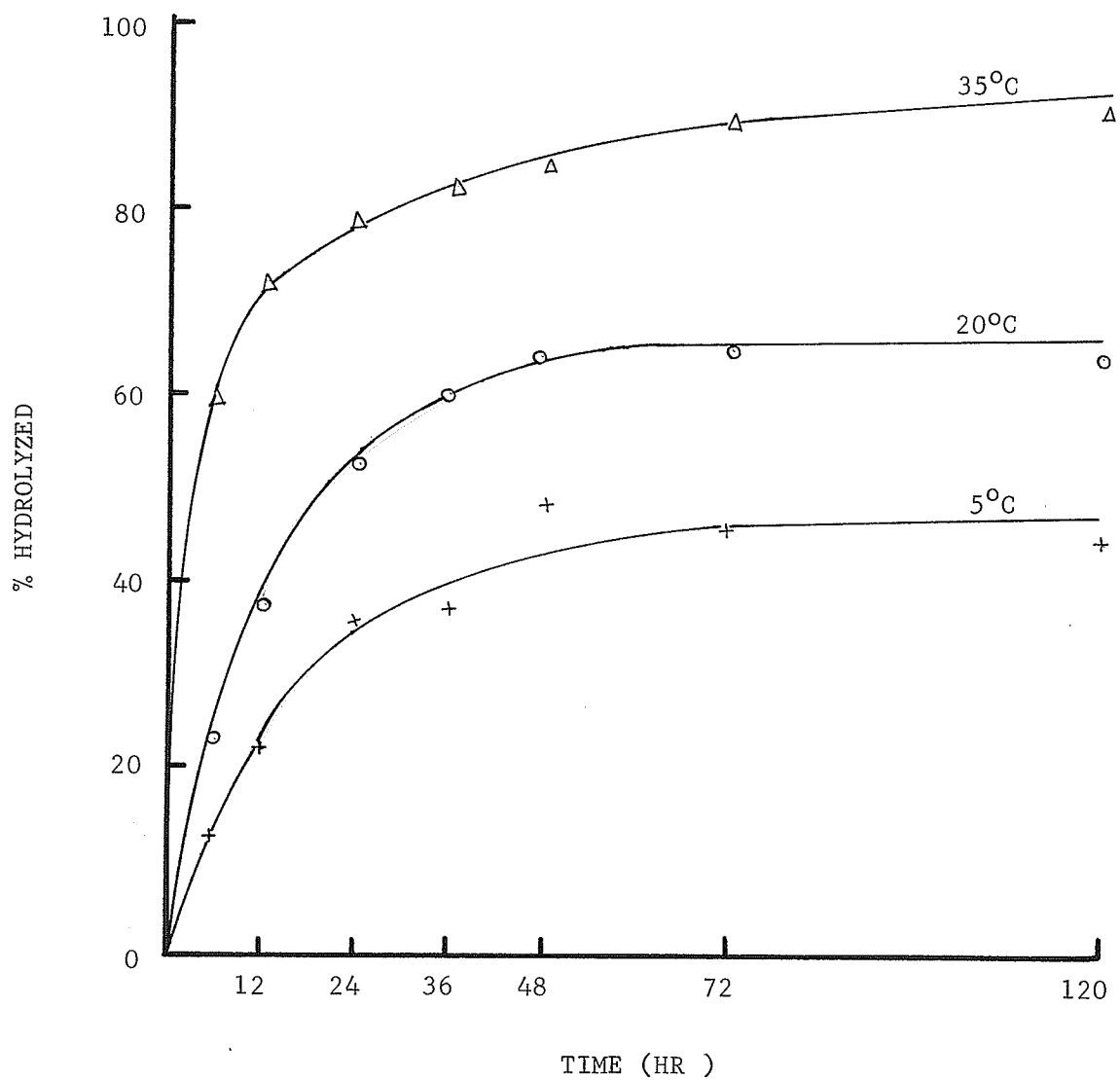
Figure 7. Effect of temperature on hydrolysis of 200 ppm-P sodium pyro-
phosphate added to the Lakeland soil--water extractable.
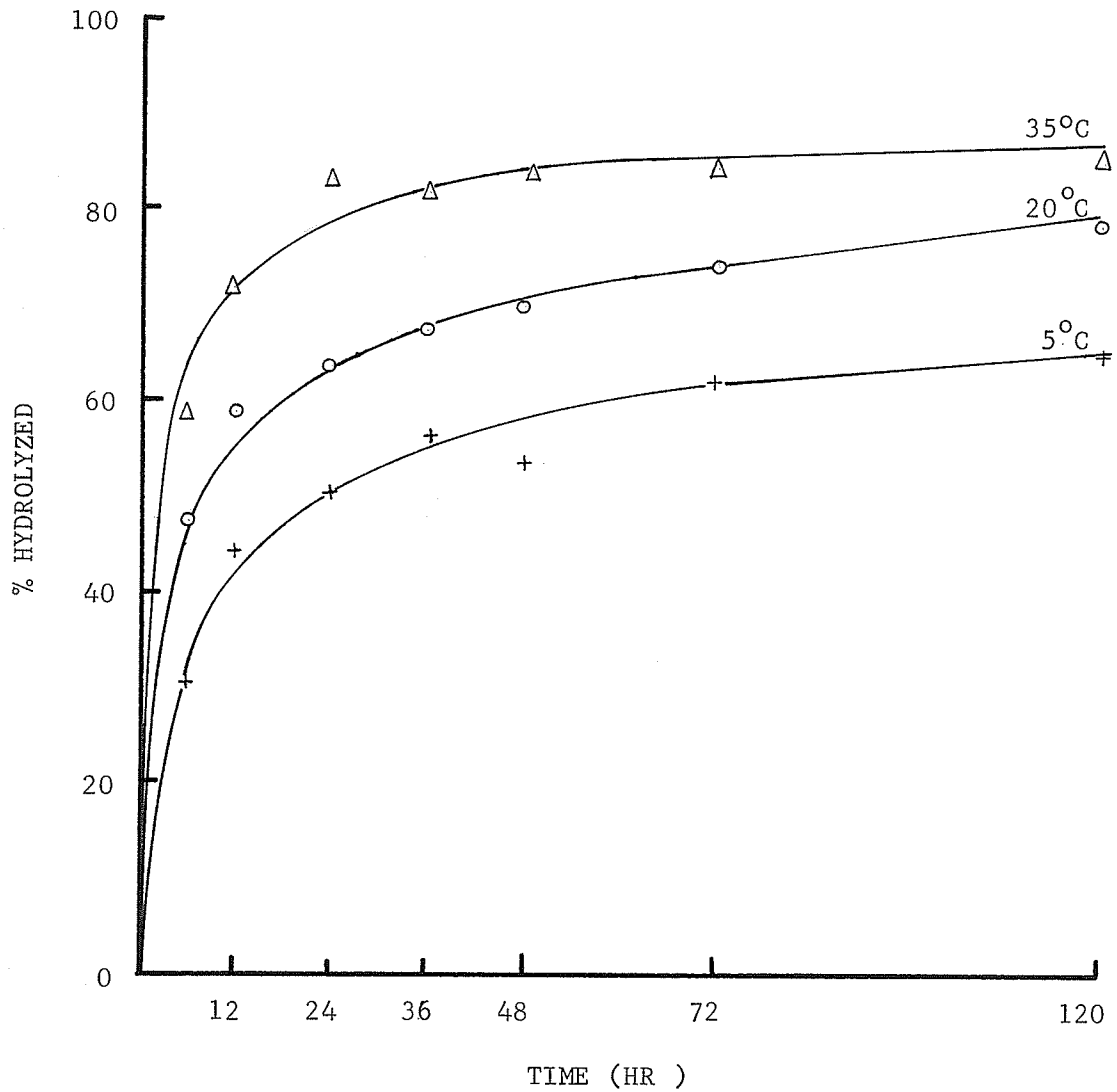
Figure 8. Effect of temperature on hydrolysis of 200 ppm-P sodium pyro-
phosphate added to the Newdale soil--water extractable.
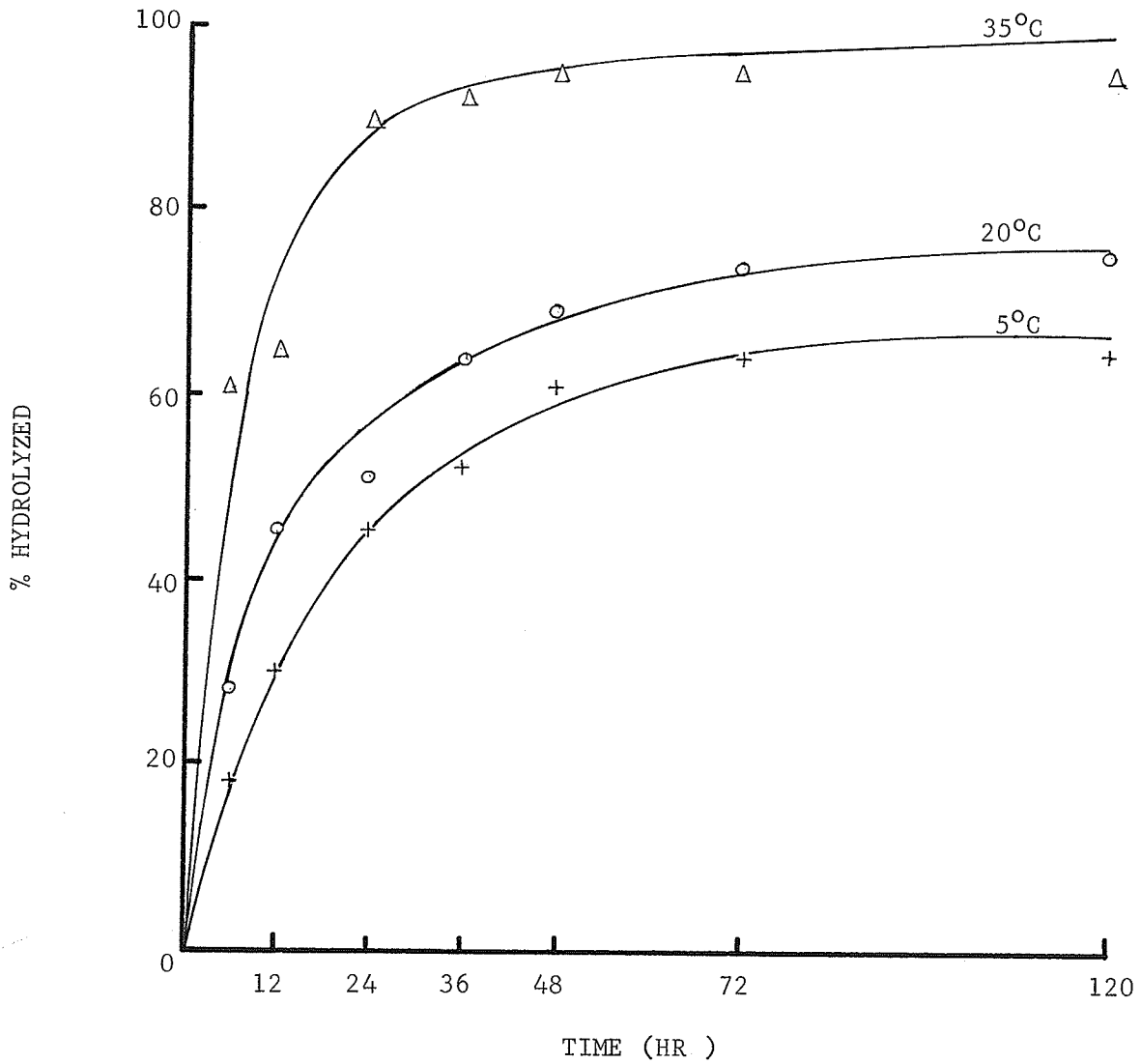
Figure 9. Effect of temperature on hydrolysis of 200 ppm-P sodium tri-
polyphosphate added to the Lakeland soil--water extractable.
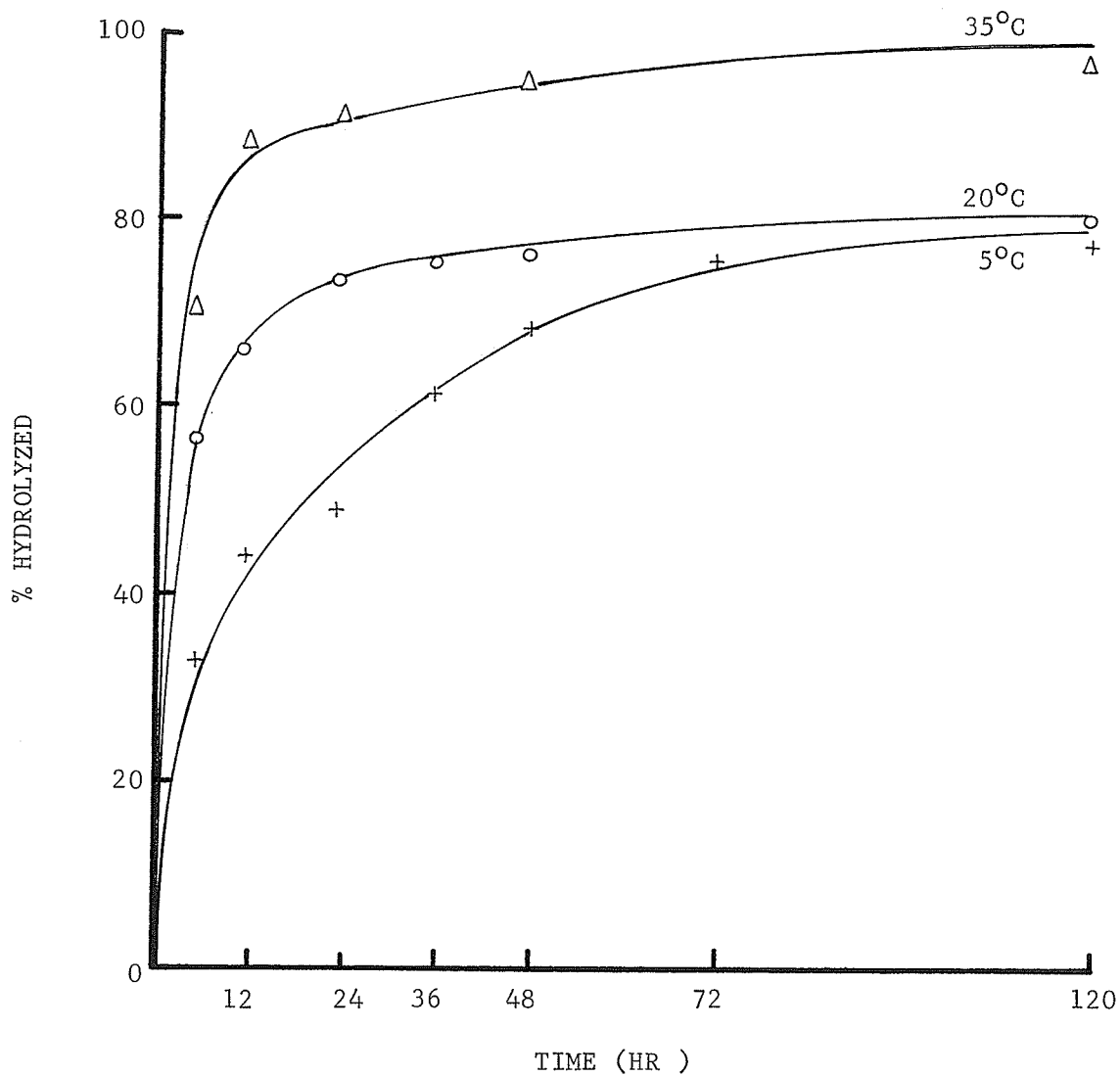
Figure 10. Effect of temperature on hydrolysis of 200 ppm-P sodium tri-
polyphosphate added to the Newdale soil--water extractable.

increased with increasing temperature. Considerably less phosphate

hydrolyzed at $5^{\circ}C$ than at 20° or $35^{\circ}C$. The influence of temperature on

rate of hydrolysis of the phosphates is also shown in Figure 11. Figure

11 was constructed by plotting the rate of hydrolysis obtained during

the first 12 hours of incubation versus temperature. The rate, $\frac{dP}{dt}$,

was calculated from the following expression: amount of orthophosphate

extracted/amount of total phosphorus extracted X 100/12 hours. The rate

of hydrolysis was found to be a linear function of temperature. The rate

of hydrolysis of both phosphates was greater in the Newdale soil than in

the Lakeland soil. In general, there probably was a higher biological

activity in the Newdale soil than in the Lakeland soil, as the rates of

hydrolysis of pyrophosphate and tripolyphosphate in the Newdale soil was

greater than in the Lakeland soil at all temperatures studied.

A preliminary plot of the logarithm of pyrophosphate concen-

tration versus time indicated that the degradation curve was represented

by two semi-logarithm linear lines. An attempt was therefore made to des-

cribe, by the use of equations, the degradation of pyrophosphate in soil.

Some workers (Thorup and Mehlich, 1961; Sutton and Larsen, 1964) have

shown that added pyrophosphate or tripolyphosphate not only undergoes

hydrolysis in soil but also is adsorbed or precipitated in soils. It

has also been shown that adsorption or precipitation of the phosphates

greatly retards their rate of hydrolysis (Savant and Racz, 1971a). Thus,

if the assumption is made that the major part of hydrolysis takes place

in the soil solution, the following diagram would represent the fate

of pyrophosphate in soil:
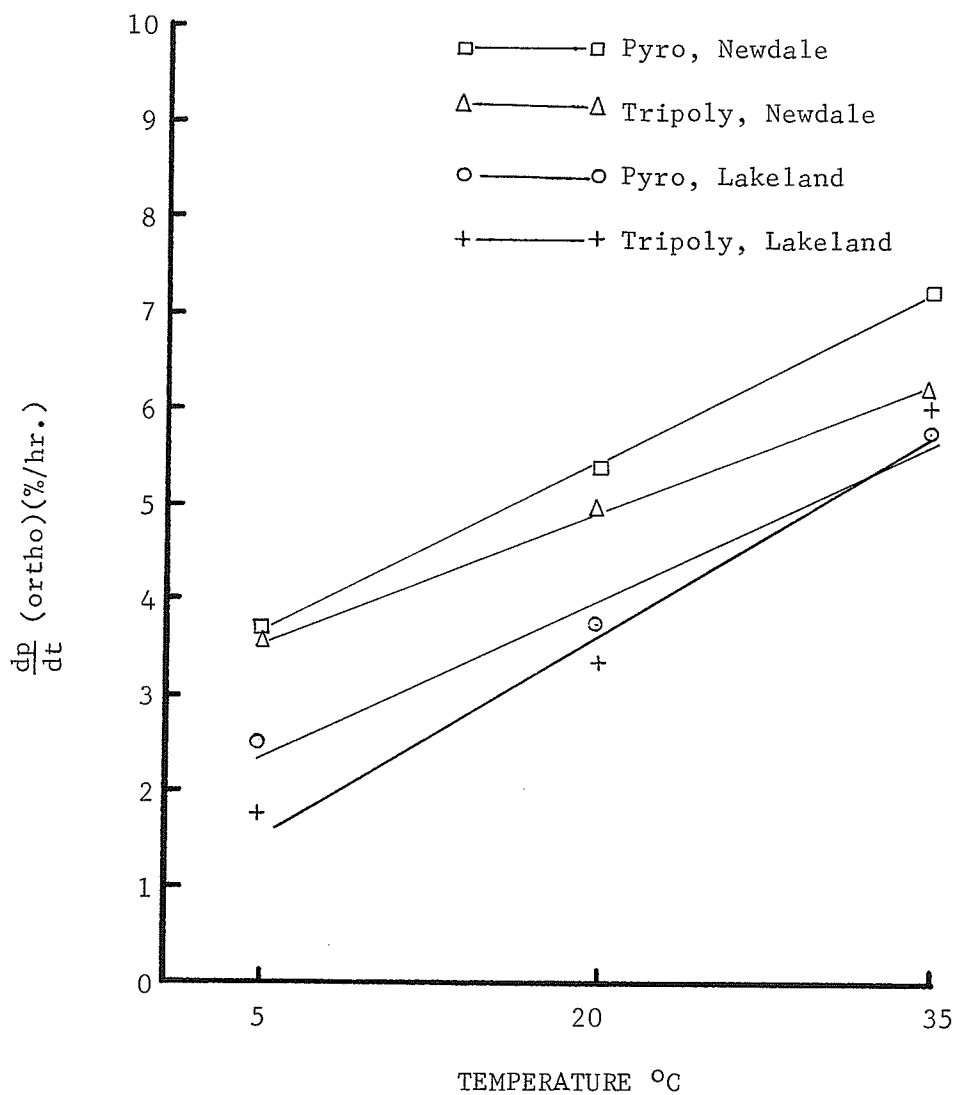
Figure 11. Effect of temperature on rate of hydrolysis, $(\frac{dP}{dt})$, of 200 ppm-P

sodium pyrophosphate and sodium tripolyphosphate in soil-
water extractable.

$$P_2O_7^{4-} \xrightarrow{\quad \text{hydrolysis} \quad} PO_4^{3-}$$

(solution)

sorption or
precipitation

Soil

desorption or dissolution
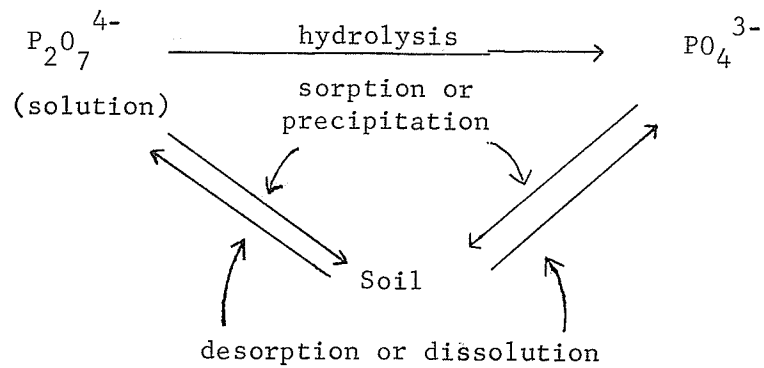
The rate of change in pyrophosphate concentration was assumed to follow the following kinetic equation:

$$- \frac{dP_2}{dt} = k_2 P_2 + k_2' P_2 (1-\theta_2) - k'_{-2} \theta_2 \qquad (18)$$

The change in the fraction of adsorption sites, θ_2, occupied by pyrophosphate is given by the equation:

$$\frac{d\theta_2}{dt} = k_2' P_2 (1-\theta_2) - k'_{-2} \theta_2 \qquad (19)$$

The rate of change in orthophosphate in the soil solution is assumed to be given by the equation:

$$\frac{dP_1}{dt} = 2k_2 P_2 - k_1' P_1 (1-\theta_2) + k'_{-1} \theta_1 \qquad (20)$$

The rate of change of the fraction of adsorption sites occupied by orthophosphate is given by the equation:

$$\frac{d\theta_1}{dt} = k_1' P_1 (1-\theta_1) - k'_1 \theta_1 \qquad (21)$$

where P_2 is the concentration of water extractable pyrophosphate in moles/l at any time,

θ_2 the fraction of the sites occupied by adsorbed pyrophosphate,

k_2' and k'_{-2} the rate constant of adsorption and desorption of

pyrophosphate, respectively,

P_1 the concentration of water extractable orthophosphate in moles/l at various times,

θ_1 the fraction of the sites occupied by adsorbed orthophosphate,

k_1' and k_{-1}' the constant of adsorption and desorption of ortho- phosphate, respectively,

and t the time the concentration of orthophosphate was recorded. When t is not too large and the total phosphates in the system is small, the value of θ_2 and θ_1 are much smaller than one and equations (18) and (19) can be approximated as

$$- \frac{dP_2}{dt} = (k_2 + k_2')\ P_2 - k_{-2}\ \theta_2 \tag{22}$$

$$\frac{d\theta_2}{dt} = k_2' P_2 - k_{-2}'\ \theta_2 \tag{23}$$

The solution of P_2 is:

$$P_2 = A_1\ e^{r_1 t} + A_2\ e^{r_2 t} \tag{24}$$

where, $r_1 = \dfrac{-(k_2 + k_2' + k_{-2}') + \sqrt{(k_2 + k_2' + k_{-2}')^2 - 4k_2 k_{-2}'}}{2}$,

$r_2 = \dfrac{-(k_2 + k_2' + k_{-2}') - \sqrt{(k_2 + k_2' + k_{-2}')^2 - 4k_2 k_{-2}'}}{2}$,

$A_1 = \dfrac{P_2^{\,o}\ (r_2 - k_2 - k_2')}{r_2 - r_1}$,

$A_2 = \dfrac{P_2^{\,o}\ (k_2 + k_2' - r_1)}{r_2 - r_1}$,

P_2, k_2, k_2', k_{-2}' and t are as indicated before, and

$P_2^{\,o}$ the initial concentration of pyrophosphate in mole/l.

If the concentration units are changed from moles/l to ppm-P, equation (24) is modified as follows:

$$P_2^* = A_1^* e^{r_1 t} + A_2^* e^{r_2 t} \qquad (25)$$

where P_2^* is the pyrophosphate concentration in ppm-P at anytime,

$$A_1^* = \frac{P_2^{o*}(r_2 - k_2 - k_2')}{r_2 - r_1} \quad ,$$

$$A_2^* = \frac{P_2^{o*}(k_2 + k_2' - r_1)}{r_2 - r_1} \quad ,$$

and P_2^{o*} the initial pyrophosphate concentration in ppm-P.

Assuming that equation (24) represented the experimental curves, the values of A_1^*, A_2^*, r_1 and r_2 of equation (25) were obtained using the experimental results shown in Tables 7 and 8. At any time following the initial rapid rate of pyrophosphate hydrolysis, the term $A_2^* e^{r_2 t}$ is smaller than the term $A_1^* e^{r_1 t}$ in equation (25) and therefore

$$P_2^* = A_1 e^{r_1 t}$$

$$\text{or} \quad \ln P_2^* = r_1 t + \ln A_1^* \qquad (26)$$

Using equation (26), r_1 and $\ln A_1^*$ can be calculated from a plot of $\ln P_2^*$ versus t. During the initial stages of the reaction (rapid reaction rate) both terms in equation (25) need be considered in solving for P_2^*. Thus

$$P_2^* - A_1^* e^{r_1 t} = A_2^* e^{r_2 t}$$

$$\text{or} \quad \ln(P_2^* - A_1^* e^{r_1 t}) = r_2 t + \ln A_2^* \qquad (27)$$

where $A_1^* e^{r_1 t}$ is obtained from equation (26). $\ln A_2^*$ and r_2 can be calculated from a plot of $\ln(P_2^* - A_1^* e^{r_1 t})$ versus t. The values obtained for r_1, A_1^*, r_2 and A_2^* using equations (26) and (27) were substituted into equation (25). The equations obtained for the degradation of

pyrophosphate in the Lakeland and Newdale soils are shown below:

Lakeland soil at 5°C

$$P_2^* = 31.78 \text{ X } e^{-0.1338t} + 9.46 \text{ X } e^{-1.1686}$$

at 20°C.

The datum for the six-hour incubation for the Lakeland soil appeared to be in error. Thus calculations were made with and without the six-hour incubation. The first equation below was calculated using all the data, whereas the second equation was calculated following exclusion of the datum obtained for the six-hour incubation.

$$P_2^* = 18.99 \text{ X } e^{-0.0583t} + 23.24 \text{ X } 10^{-3.8716t}$$

$$P_2^* = 18.99 \text{ X } e^{-0.0583t} + 8.92 \text{ X } 10^{-1.6704t}$$

at 35°C

$$P_2^* = 8.11 \text{ X } e^{-0.2288t} + 7.06 \text{ X } e^{-1.2908t}$$

Newdale soil at 5°C.

$$P_2^* = 28.74 \text{ X } e^{-0.0426t} + 20.48 \text{ X } e^{-2.4561t}$$

at 20°C

$$P_2^* = 22.30 \text{ X } e^{-0.0330t} + 15.04 \text{ X } e^{-3.7278t}$$

at 35°C

$$P_2^* = 14.75 \text{ X } e^{-0.0659t} + 19.63 \text{ X } e^{-3.3615t}$$

In order to obtain the rate constant corresponding to the hydrolysis of pyrophosphate during the initial stages of reaction, equation (24) expressed in logarithmic form was differentiated and the limiting value at t = o taken as follows:

$$\frac{d\ln P_2^*}{dt} = \frac{r_1 A_1^* e^{r_1 t} + r_2 A_2^* e^{r_2 t}}{A_1^* e^{r_1 t} + A_2^* e^{r_2 t}} \tag{28}$$

$$\lim_{t=o} \frac{d\ln P_2}{dt} = \frac{r_1 A_1^* + r_2 A_2^*}{A_1^* + A_2^*} \tag{29}$$

Therefore the initial slope of the exponential degradation equation is $\frac{r_1 A_1^* + r_2 A_2^*}{A_1^* + A_2^*}$ during the initial stages of reaction and is r_2 at the later stages.

The influence of temperature on the initial rapid rate of reactions (hydrolysis, sorption and desorption) of pyrophosphate in soil is shown in Figure 12 for the Lakeland and Newdale soils. The natural logarithm of $\frac{r_1 A_1^* + r_2 A_2^*}{A_1^* + A_2^*}$ was plotted versus the reciprocal of the absolute temperature. A straight line relationship was obtained for both soils when, the datum for the six-hour incubation was excluded from the calculations for r_2, A_2^* and $\ln \frac{r_1 A_1^* + r_2 A_2^*}{A_1^* + A_2^*}$ for the Lakeland soil at 20°C. If this data were considered the value for $\ln \frac{r_2 A_1^* + r_2 A_2^*}{A_1^* + A_2^*}$ was extremely large and above that for the Newdale soil. Since the Lakeland soil usually hydrolyzed pyrophosphate at a slower rate than did the Newdale soil, exclusion of the data may be justified.

The reactions of tripolyphosphate in soil is more complicated than that of pyrophosphate. If the assumption is made that the sorbed or precipitated tripolyphosphate does not contribute greatly to the rate of tripolyphosphate hydrolysis, the fate of tripolyphosphate in soil can be shown as follows:
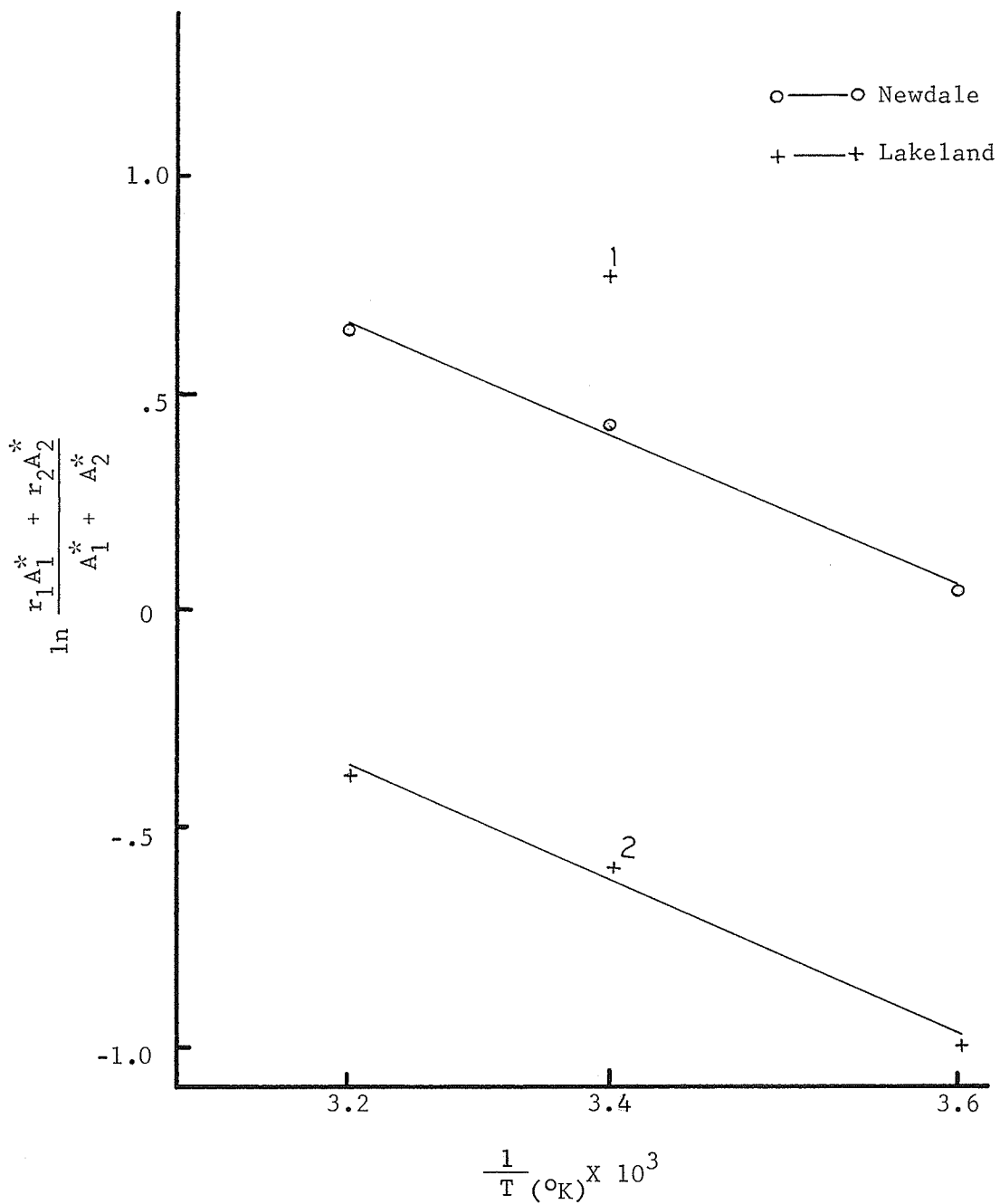
1. Includes datum obtained for the 6-hr. incubation.
2. Excludes datum obtained for the 6-hr. incubation.

Figure 12. Variation of natural logarithm of $\dfrac{r_1A_1^* + r_2A_2^*}{A_1^* + A_2^*}$ with reciprocal of temperature (1/T).

$$P_3O_{10}^{5-} \xrightarrow{\text{hydrolysis}} P_2O_7^{4-}$$

$$PO_4^{3-}$$

hydrolysis

sorption or precipitation

desorption or dissolution

desorption or dissolution

sorption or precipitation

sorption or precipitation

desorption or dissolution

Soil
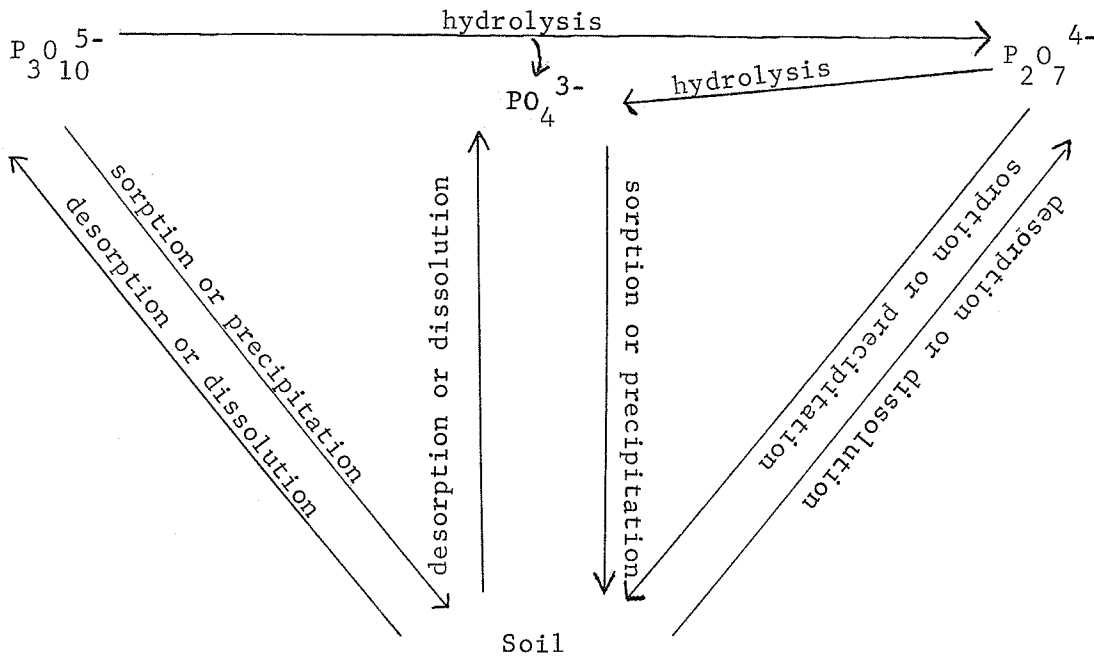
If the above scheme is selected for this complex system, the following rate equation can be tentatively written:

$$-\frac{dP_3}{dt} = k_3 P_3 + k_3' P_3 (1-\theta_3) - k_{-3}' \theta_3 \qquad (30)$$

The rate of change in the fraction of adsorption sites, θ_3, occupied by tripolyphosphate is given by the equation:

$$\frac{d\theta_3}{dt} = k_3' P_3 (1 - \theta_3) - k_{-3}' \theta_3 \qquad (31)$$

The rate of formation of pyrophosphate in soil solution is given by:

$$\frac{dP_2}{dt} = k_3 P_3 - k_2 P_2 - k_2' P_2 (1 - \theta_2) + k_{-2}' \theta_2 \qquad (32)$$

The rate of change in the fraction of adsorption sites, θ_2, occupied by pyrophosphate is given by the equation:

$$\frac{d\theta_2}{dt} = k_2' P_2 (1 - \theta_2) - k_{-2}' \theta_2 \qquad (33)$$

The rate of appearance of orthophosphate in the soil solution is given

by the equation:

$$\frac{dP_1}{dt} = k_3P_3 + 2k_2P_2 - k_1'P_1(1 - \theta_1) + k_{-1}'\theta_1 \qquad (34)$$

The rate of change in the fraction of adsorption sites, θ_1, occupied by orthophosphate is given by the equation:

$$\frac{d\theta_1}{dt} = k_1'P_1(1 - \theta_1) - k_{-1}'\theta_1 \qquad (35)$$

Where, P_3 is the concentration of extractable tripolyphosphate in moles/1 at any time,

k_3 the rate constant of hydrolysis of tripolyphosphate,

k_3' and k_{-3}' the rate constants of sorption and desorption of tripolyphosphate, respectively,

θ_3 the fraction of the sites occupied by adsorbed tripolyphosphate,

and P_2, P_1, k_2, k_2', k_{-2}', k_1' and k_{-1}' are as defined for pyrophosphate.

However, it is not possible to solve for P_1 or obtain even a simple approximate solution, as only the total and orthophosphate concentrations in soil solution was measured. Thus equations such as were calculated for pyrophosphate hydrolysis could not be calculated for tripolyphosphate hydrolysis in soil.

Experiment 2. The influence of temperature on the rate of sodium pyrophosphate hydrolysis in soil.

A second set of incubations was conducted to further investigate the influence of temperature on the rate of hydrolysis of pyrophosphate. In addition to studying hydrolysis at 5, 20 and 35°C, samples were also incubated at temperatures of 50 and 65°C. Only sodium pyrophosphate was added. Both the Lakeland and Newdale soils were selected

for study. The hydrolysis of water extractable phosphorus was studied for both soils. The hydrolysis of 0.5N H_2SO_4 extractable phosphorus was studied using only the Newdale soil.

The data on the hydrolysis of water extractable pyrophosphate are shown in Tables 11 and 12. The recovery of added phosphorus varied with time of incubation and approximated those found for the first experiment. The amount of orthophosphate extracted from the soils increased with time of incubation. The amount of pyrophosphate extracted decreased with time of incubation and incubation temperature.

The amounts of total phosphorus, orthophosphate and pyrophosphate extracted by 0.5N H_2SO_4 from the Newdale soil are shown in Table 13. The sulfuric acid usually extracted more of the added phosphorus than did water. The influence of time and temperature of incubation on the amounts of orthophosphate and pyrophosphate extracted by 0.5N H_2SO_4 followed trends noted for these phosphates extracted by water. The total recovery of added phosphate by water and 0.5N H_2SO_4 varied from 60 to 90%.

The rates of hydrolysis of water extractable pyrophosphate in this experiment were much slower than those found for pyrophosphate in the first experiment (Figures 13 and 14). Since the soils were stored in an air-dried condition for approximately six months prior to initiation of the second experiment, it is possible that the enzymatic activity of the soils was reduced by storage (Jackman and Black, 1952). The soils were not stored prior to initiation of the first experiment. The optimum temperature for hydrolysis of pyrophosphate in the Newdale soil was 50°C (Figure 14). This corresponds well with the finding that 50°C is the optimum temperature for phosphatase activity and that above this

Table 11

Effect of incubation temperature and time on the hydrolysis of
pyrophosphate extracted by water from the Lakeland soil
treated with 200 ppm-P pyrophosphate

Incubation temperature (°C)		Phosphorus extracted (ppm-P) at various times of incubation			
		6 hours	12 hours	24 hours	36 hours
5	total phosphorus	35.0	34.0	29.0	29.0
	orthophosphate	5.0	6.0	6.1	10.0
	pyrophosphate	30.0	28.0	22.9	19.0
20	total phosphorus	31.0	32.3	33.0	-*
	orthophosphate	5.0	7.2	13.2	-*
	pyrophosphate	26.0	25.0	19.8	-*
35	total phosphorus	34.0	27.0	27.0	26.3
	orthophosphate	5.8	7.0	14.9	18.4
	pyrophosphate	28.2	20.0	12.1	7.9
50	total phosphorus	44.3	41.7	43.3	43.3
	orthophosphate	8.2	13.6	21.0	21.0
	pyrophosphate	36.1	28.1	22.3	22.3
65	total phosphorus	25.3	27.0	28.3	28.3
	orthophosphate	14.2	21.0	28.0	27.0
	pyrophosphate	11.1	6.0	3.0	1.3

* Sample lost during extraction.

Table 12

Effect of incubation temperature and time on the hydrolysis of pyrophosphate extracted by
water from the Newdale soil treated with 200 ppm-P pyrophosphate

Incubation temperature (°C)		Phosphorus extracted (ppm-P) at various times of incubation				
		6 hours	24 hours	48 hours	168 hours	336 hours
5	total phosphorus	-*	57.5	51.2	42.8	44.5
	orthophosphorus	-*	19.0	25.0	30.0	36.0
	pyrophosphate	-*	38.5	26.2	12.8	8.5
20	total phosphorus	65.0	67.5	61.5	47.3	44.5
	orthophosphorus	25.4	29.0	34.0	35.0	36.3
	pyrophosphate	49.6	48.5	37.5	12.3	8.2
35	total phosphorus	75.5	68.5	60.5	48.7	43.5
	orthophosphorus	30.0	38.0	42.0	43.0	43.5
	pyrophosphate	45.5	30.5	18.5	2.7	0.0
50	total phosphorus	-*	71.0	57.5	44.4	37.5
	orthophosphorus	-*	55.0	51.0	43.0	37.0
	pyrophosphate	-*	16.0	6.5	1.4	0.5
65	total phosphorus	75.0	71.0	71.7	48.0	46.0
	orthophosphorus	26.0	40.0	45.4	39.0	40.4
	pyrophosphate	49.0	31.0	26.3	9.0	6.4

* Sample lost during extraction.
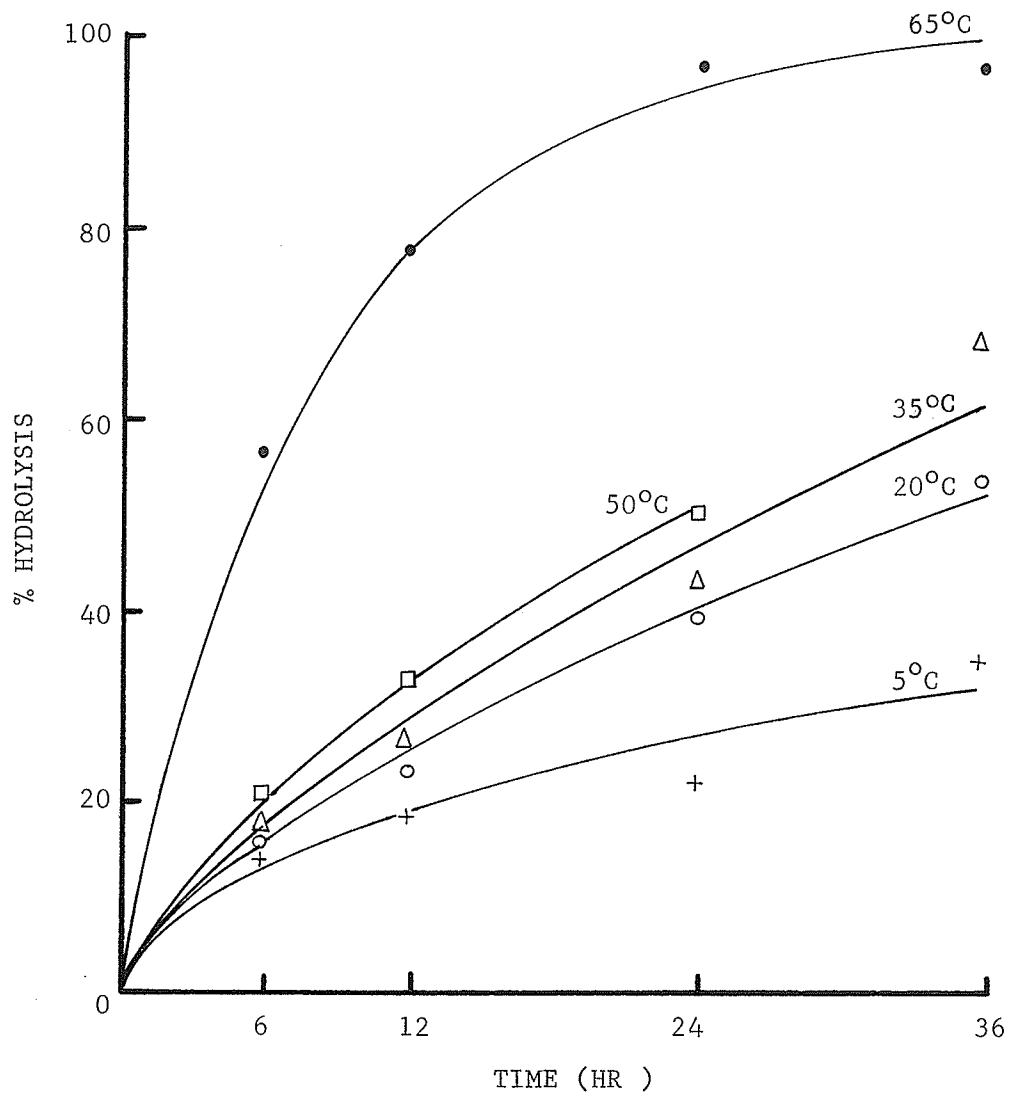
Figure 13. Effect of temperature on hydrolysis of 200 ppm-P sodium pyrophosphate added to the Lakeland soil-water extractable.
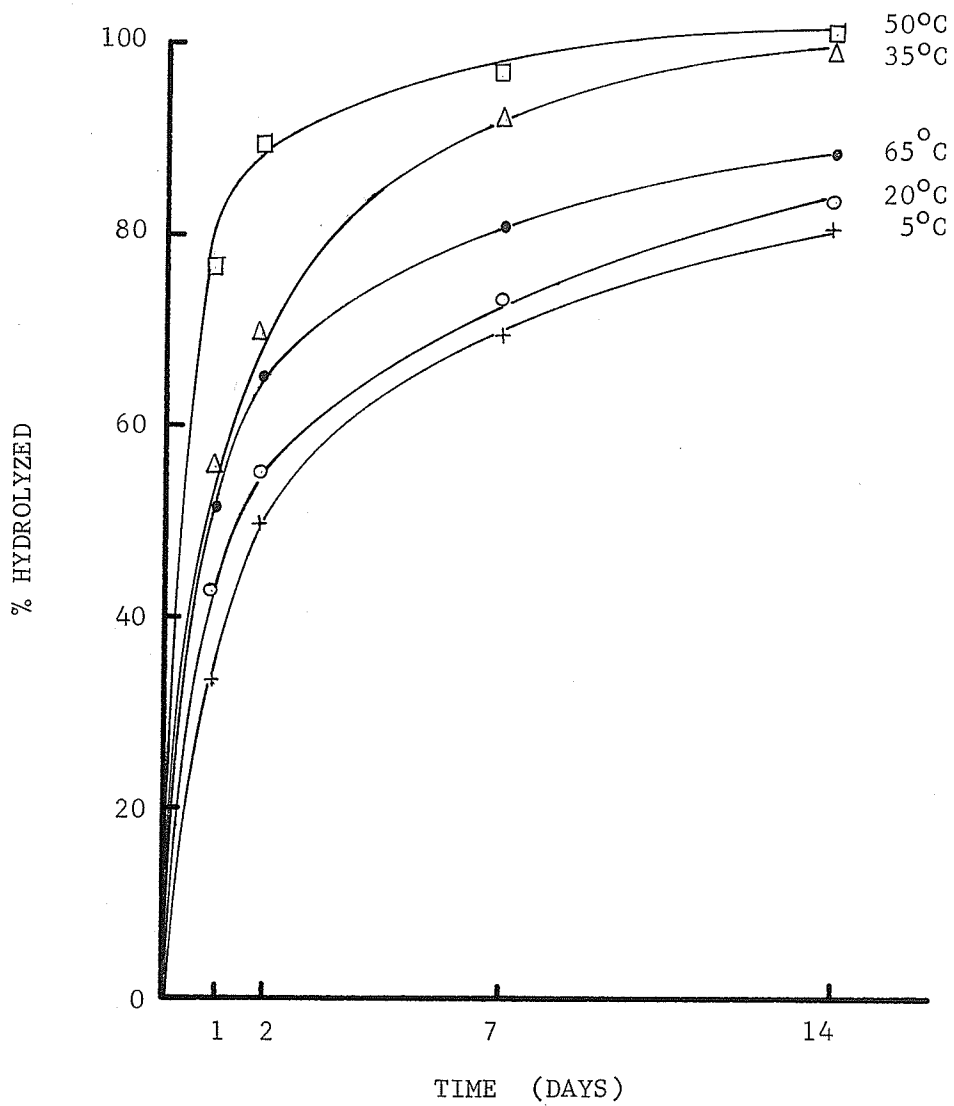
Figure 14. Effect of temperature on hydrolysis of 200 ppm-P sodium pyro-
phosphate added to the Newdale soil--water extractable.

Table 13

Effect of incubation temperature and time on the hydrolysis of pyrophosphate extracted by
0.5N H_2SO_4[**] from the Newdale soil treated with 200 ppm-P pyrophosphate

Incubation temperature (°C)		Phosphorus extracted (ppm-P) at various times of incubation				
		6 hours	24 hours	48 hours	168 hours	336 hours
5	total phosphorus	-*	94.0	90.0	75.0	70.0
	orthophosphate	-*	4.5	24.0	34.0	47.6
	pyrophosphate	-*	89.5	66.0	41.0	22.4
20	total phosphorus	92.0	90.0	90.0	72.0	70.0
	orthophosphate	11.0	25.0	61.0	63.0	69.3
	pyrophosphate	81.0	65.0	19.0	9.0	0.3
35	total phosphorus	77.0	90.0	101.0	114.0	123.0
	orthophosphate	11.0	30.0	53.0	80.0	105.0
	pyrophosphate	66.0	60.0	48.0	34.0	18.0
50	total phosphorus	-*	110.0	112.0	117.0	136.0
	orthophosphate	-*	51.3	65.0	87.0	125.0
	pyrophosphate	-*	58.7	47.0	30.0	11.0
65	total phosphorus	59.0	56.0	113.3	125.5	145.0
	orthophosphate	7.6	29.6	65.6	98.0	122.0
	pyrophosphate	51.4	26.4	47.7	27.5	23.0

* Sample lost during extraction.

** After water extraction.

temperature the enzyme begins to break down (Roche, 1950). However, in the Lakeland soil, the greatest hydrolysis rate was recorded at 65°C (Figure 13). It is possible that some chemical hydrolysis may have occurred at this high temperature which more than compensated for a decrease in enzyme activity.

The work of Savant and Racz (1971a) showed that the strongly absorbed or precipitated pyrophosphate hydrolyzed at a slower rate than pyrophosphate which is not or only weakly fixed by the soil. The percentage of orthophosphate appearing in the $0.5N$ H_2SO_4 extractable fraction was less than that in the water extractable fraction (Figures 14 and 15) even though the concentration of orthophosphate in the former fraction was greater (Tables 12 and 13). This indicates that pyrophosphate which is strongly adsorbed or precipitated by soil hydrolyzed at a slower rate than pyrophosphate which is not or only weakly fixed by the soil. The rate and extent of pyrophosphate hydrolysis increased with increasing temperature except for the water and sulfuric acid extractable phosphorus at 65°C in the Newdale soil. The influence of temperature on the rate of hydrolysis of pyrophosphate is also shown in Figure 16 in which the rate after 12, or 24 hours of incubation is plotted versus temperature. The rates of hydrolysis in both soils were a linear function of temperature at temperatures of 5 to 50°C. Variable results were obtained above a temperature of 50°C. The rates of hydrolysis of water extractable pyrophosphate in both soils were similar. However, phosphate extracted by $0.5N$ H_2SO_4 appeared to hydrolyze at a much slower rate than did phosphate extracted with water. Although $0.5N$ H_2SO_4 extractable phosphate hydrolyzed at a slower rate than water extractable phosphate the influence of temperature on both forms of extractable phosphorus was
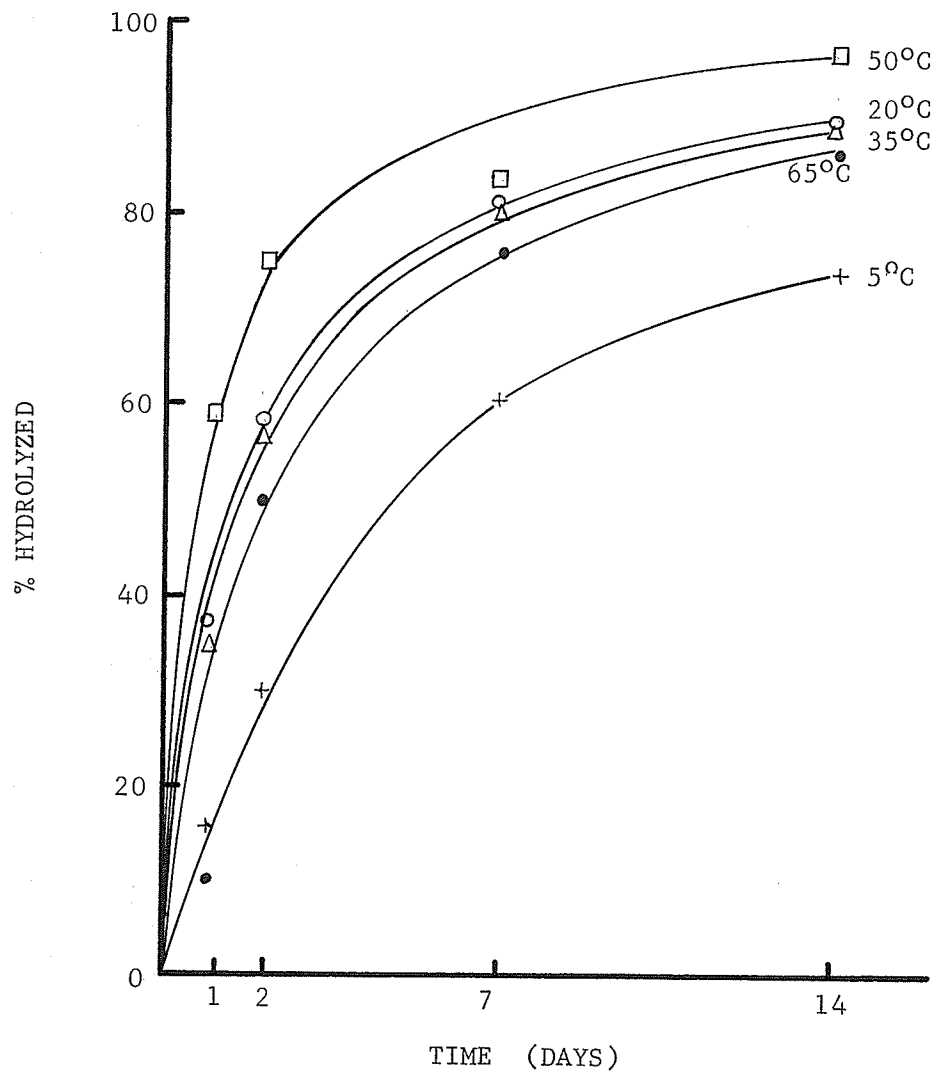
Figure 15. Effect of temperature on hydrolysis of 200 ppm-P sodium pyro-
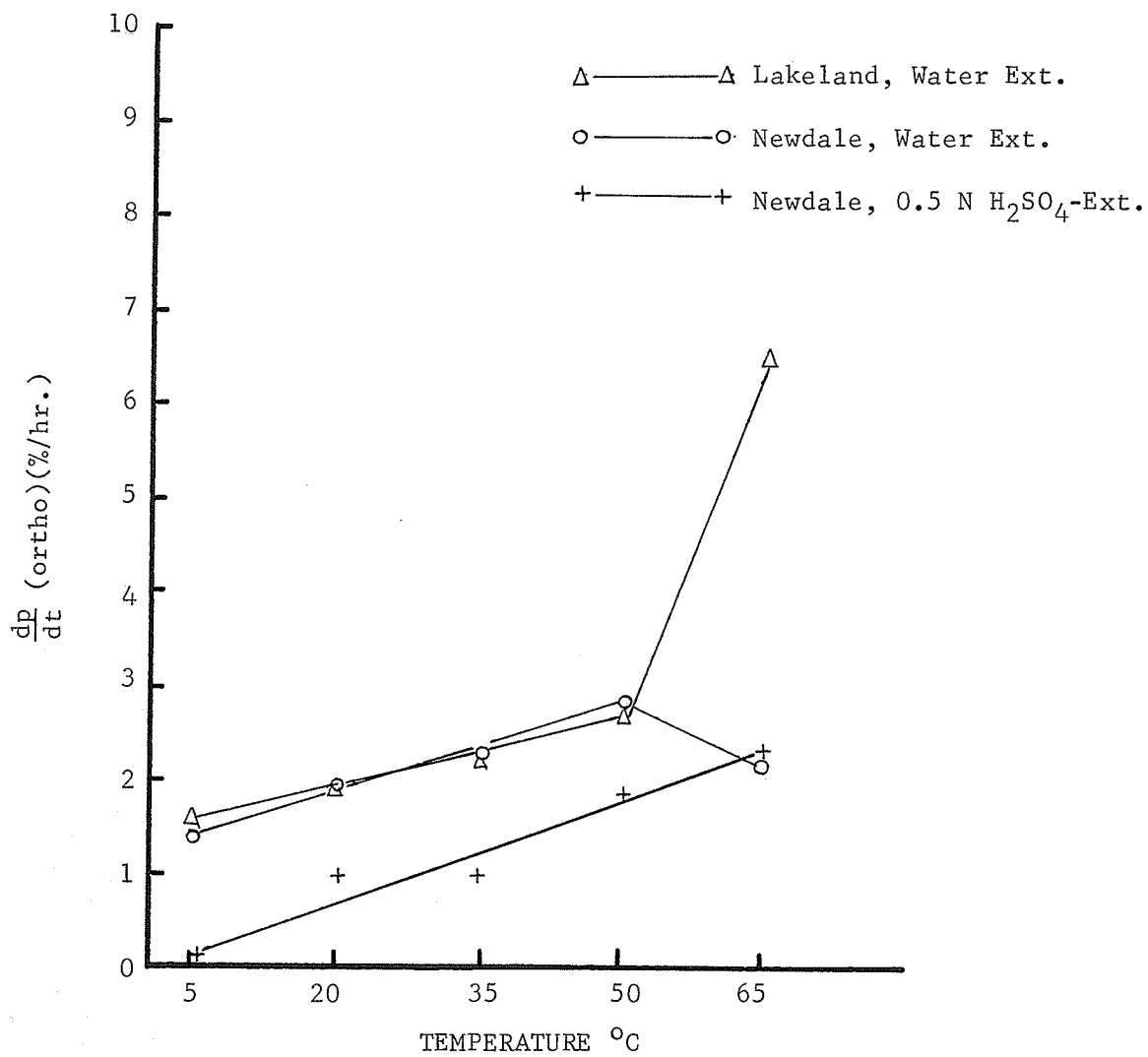phosphate added to the Newdale soil--0.5N H_2SO_4 extractable.

Figure 16. Effect of temperature on rate of hydrolysis of sodium pyro-
phosphate extracted by water and 0.5N H_2SO_4.

similar.

Since enzymatic activity appeared to be greatly retarded by storage of the samples in an air-dried state, no attempt was made to calculate equations representing the degradation of pyrophosphate as was done in Experiment 1. This experiment, however, showed that the rate of hydrolysis is a linear function of temperature at temperatures of 5 to 50°C and that pyrophosphate extracted by 0.5N H_2SO_4 hydrolyzes at a much slower rate than pyrophosphate extracted by water.

Experiment 3. The influence of substrate concentration of sodium pyrophosphate and sodium tripolyphosphate hydrolysis in soil.

The amounts of orthophosphate, total phosphate and polyphosphate extracted from the Lakeland and Newdale soils treated with varying amounts of pyrophosphate and tripolyphosphate is shown in Tables 14 to 17. The amounts of total water extractable phosphorus (ppm-P) recovered increased with increased amounts of phosphate added. The percentage of added phosphate recovered also usually increased with the amount of phosphate added. This may be due to a reduction in soil phosphate fixation sites with increased rate of application. Thus, although the soils continued to fix phosphate even at high levels of added phosphorus the ratio of the phosphorus fixed by the soil to that added decreased with increasing amounts of pyrophosphate added. The amount and percentage of added phosphate extracted decreased with time of incubation when 400 ppm-P or greater was added. This was probably due to the rate of pyrophosphate and orthophosphate adsorption or precipitation being greater than the rate of pyrophosphate and orthophosphate desorption or dissolution. Recovery of tripolyphosphate was greater than the recovery of pyrophosphate. Also, more phosphate was

Table 14

Effect of incubation time and concentration of added pyrophosphate on the amounts of orthophosphate, pyrophosphate and total phosphorus extracted by water from the Lakeland soil

Rate of Phosphorus added (ppm-P)		Phosphorus extracted (ppm-P) at various times of incubation						
		6 hours	12 hours	24 hours	36 hours	48 hours	72 hours	120 hours
200	total phosphorus	35.9	35.4	39.6	43.8	46.0	46.0	48.0
	orthophosphate	7.9	14.4	20.0	26.3	29.2	30.0	30.4
	pyrophosphate	28	21.0	19.6	17.5	16.8	16.0	17.6
400	total phosphorus	144.0	139.5	137.5	130.0	120.0	117.5	105.0
	orthophosphate	20.0	32.2	37.0	41.4	44.0	50.0	51.3
	pyrophosphate	124.0	107.3	100.5	88.6	76.0	67.5	53.7
800	total phosphorus	385.0	337.5	315.0	322.5	305.0	300.0	300.0
	orthophosphate	25.0	44.0	55.0	53.3	77.5	82.5	90.0
	pyrophosphate	360.0	293.5	260.0	268.2	227.5	217.5	210.0
1,600	total phosphorus	750.0	730.5	730.0	645.7	670.0	660.0	610.0
	orthophosphate	52.5	64.0	82.5	80.0	116.0	130.0	130.0
	pyrophosphate	697.5	666.5	647.5	565.7	594.0	530.0	480.0

Table 15

Effect of incubation time and concentration of added pyrophosphate on the amounts of orthophosphate, pyrophosphate and total phosphorus extracted by water from the Newdale soil

Rate of phosphorus added (ppm-P)		Phosphorus extracted (ppm-P) at various times of incubation						
		6 hours	12 hours	24 hours	36 hours	48 hours	72 hours	120 hours
200	total phosphorus	57.2	56.4	58.7	61.0	65.5	75.0	79.8
	orthophosphate	27.0	33.0	36.7	40.0	44.9	54.8	60.9
	pyrophosphate	30.2	23.4	22.0	21.0	20.6	20.2	19.0
400	total phosphorus	187.5	187.5	187.5	174.5	127.5	172.5	154.0
	orthophosphate	49.0	52.0	62.0	66.6	74.0	82.5	84.0
	pyrophosphate	138.5	135.5	125.5	107.9	97.5	90.0	70.0
800	total phosphorus	415.0	415.0	415.0	412.5	385.0	335.0	350.0
	orthophosphate	53.5	82.5	102.0	105	122.5	122.5	135.0
	pyrophosphate	361.5	322.5	313.0	307.5	262.5	232.5	215.0
1,600	total phosphorus	759.0	740.0	735.0	725	710.0	700.0	710.0
	orthophosphate	87.5	116.0	146.0	155	180.0	220.0	235.0
	pyrophosphate	671.5	630.0	589.0	570	520.0	480.0	485.0

Table 16

Effect of incubation time and concentration of added tripolyphosphate on the amounts of orthophosphate, polyphosphate and total phosphorus extracted by water from the Lakeland soil

Rate of phosphorus added (ppm-P)		Phosphorus extracted (ppm-P) at various times of incubation						
		6 hours	12 hours	24 hours	36 hours	48 hours	72 hours	120 hours
200	total phosphorus	41.2	37.0	39.0	26.0	28.6	34.1	36.2
	orthophosphate	11.0	16.8	20.0	16.6	19.5	24.9	27.0
	polyphosphate	30.2	20.2	19.0	9.4	9.1	9.2	9.2
400	total phosphorus	164.0	133.0	135.0	127.0	107.5	107.5	85.5
	orthophosphate	22.2	35.0	42.2	45.0	48.0	55.0	56.3
	polyphosphate	141.8	98.0	92.8	82.0	59.5	52.5	29.2
800	total phosphorus	451.0	430.5	409.0	351.0	325.0	300.0	300.0
	orthophosphate	40.0	67.0	90.0	105.0	97.5	107.5	117.0
	polyphosphate	411.0	363.5	319.0	246.0	227.5	192.5	183.0
1,600	total phosphorus	1095.0	980.0	976.0	880.6	860.0	770.0	750.0
	orthophosphate	70.0	80.0	115.0	140.0	162.0	146.0	195.0
	polyphosphate	1025.0	900.0	861.0	740.6	700.0	626.0	555.0

Table 17

Effect of incubation time and concentration of added tripolyphosphate on the amounts of orthophosphate, polyphosphate and total phosphorus extracted by water from the Newdale soil

Rate of phosphorus added (ppm-P)		Phosphorus extracted (ppm-P) at various times of incubation						
		6 hours	12 hours	24 hours	36 hours	48 hours	72 hours	120 hours
200	total phosphorus	69.0	66.0	64.6	65.6	62.0	60.5	61.0
	orthophosphate	39.0	42.9	47.0	49.0	48.0	47.5	49.0
	polyphosphate	30.0	23.1	17.6	16.6	14.0	13.0	13.0
400	total phosphorus	230.0	182.5	182.5	182.5	175.0	150.0	120.0
	orthophosphate	55.0	71.00	74.0	84.0	86.5	90.0	.92.5
	polyphosphate	175.0	111.5	108.5	98.5	88.5	60.0	27.5
800	total phosphorus	530.0	472.5	445.0	415.0	409.0	360.0	345.0
	orthophosphate	82.0	119.2	137.0	140.0	158.5	180.0	192.5
	polyphosphate	447.5	353.3	308.0	275.0	250.5	180.0	152.5
1,600	total phosphorus		1055.7	1000.0	999.2	948.0	852.0	680.0
	orthophosphate	141.5	161.2	207.5	223.3	278.0	305.0	305.0
	polyphosphate	1013.5	894.5	792.5	695.9	670.0	547.0	375.0

recovered from the Newdale than from the Lakeland soil. The amounts of orthophosphate extracted increased with time of incubation and amount of phosphate added. The amounts of pyrophosphate or tripolyphosphate extracted decreased with time of incubation but increased with the amounts of phosphate added.

The percent hydrolysis (orthophosphate extracted/total phosphorus X 100) is shown in Figures 17 to 20. The rate of hydrolysis, which is the slope of the curve at various times, (% hydrolysis) decreased with increases in the amount of pyrophosphate or tripolyphosphate added. The percentage of added phosphate hydrolyzed after 120 hours of incubation also decreased with increases in the amount of phosphate added. The rate of hydrolysis in the Newdale soil was greater than that in the Lakeland soil. Also, tripolyphosphate hydrolyzed at a slightly greater rate than did pyrophosphate. The influence of substrate concentration on the rates of hydrolysis is also shown in Figure 21 where the rate of hydrolysis, measured after 12 hours of incubation, is plotted versus substrate concentration. The rate of hydrolysis was calculated as follows: orthophosphate extracted/total phosphorus extracted X $\frac{100}{12}$. The rate of hydrolysis (% hydrolysis) decreased with increases in the amount of phosphate added. However, the decreases in rate with increases in added phosphate at high phosphorus concentrations was less than that at low phosphorus concentrations. Although the rate, expressed as % hydrolysis, decreased with increases in substrate concentration, the amount (ppm) of orthophosphate extracted from the soils increased with increases in the amount of phosphorus added (Tables 14 to 17). The effect of substrate concentration on the rate of orthophosphate produced during 12 hours of incubation is shown in Figure 22. The rate of
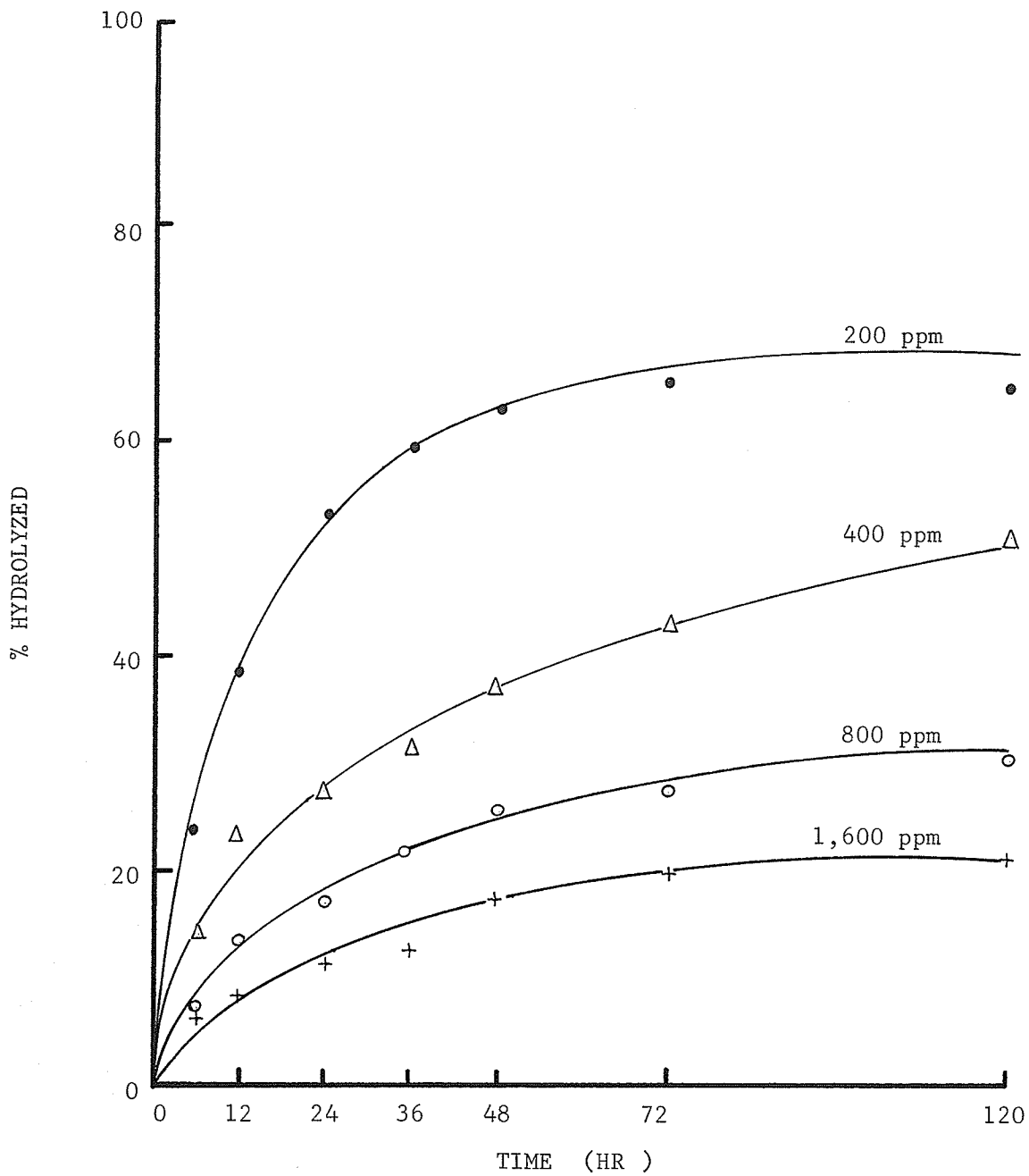
Figure 17. The influence of substrate concentration on the percent hy-
            drolysis of sodium pyrophosphate added to the Lakeland soil--
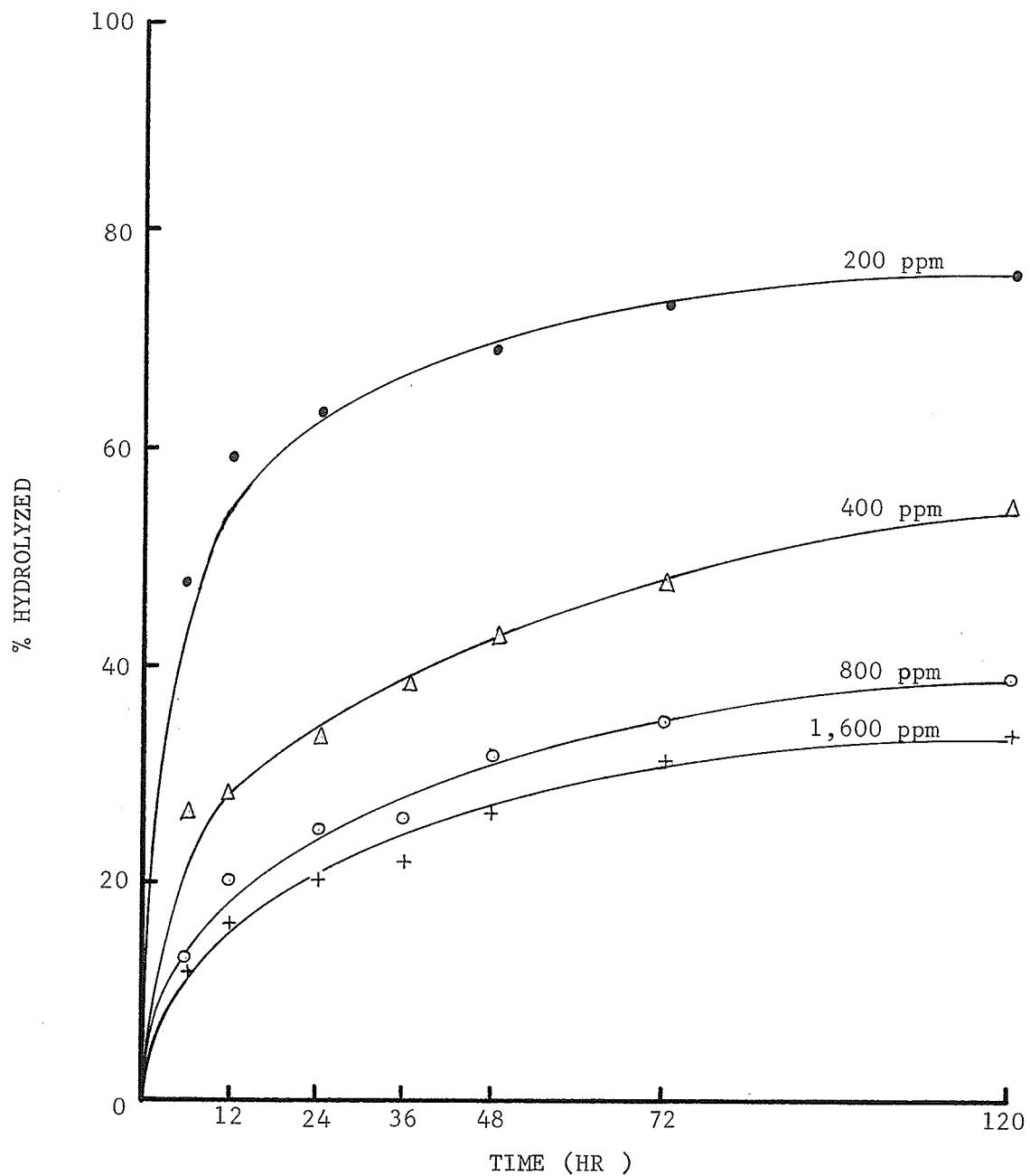   water extractable.

Figure 18. The influence of substrate concentration on the percent hydrolysis
of sodium pyrophosphate added to the Newdale soil--water extractable.
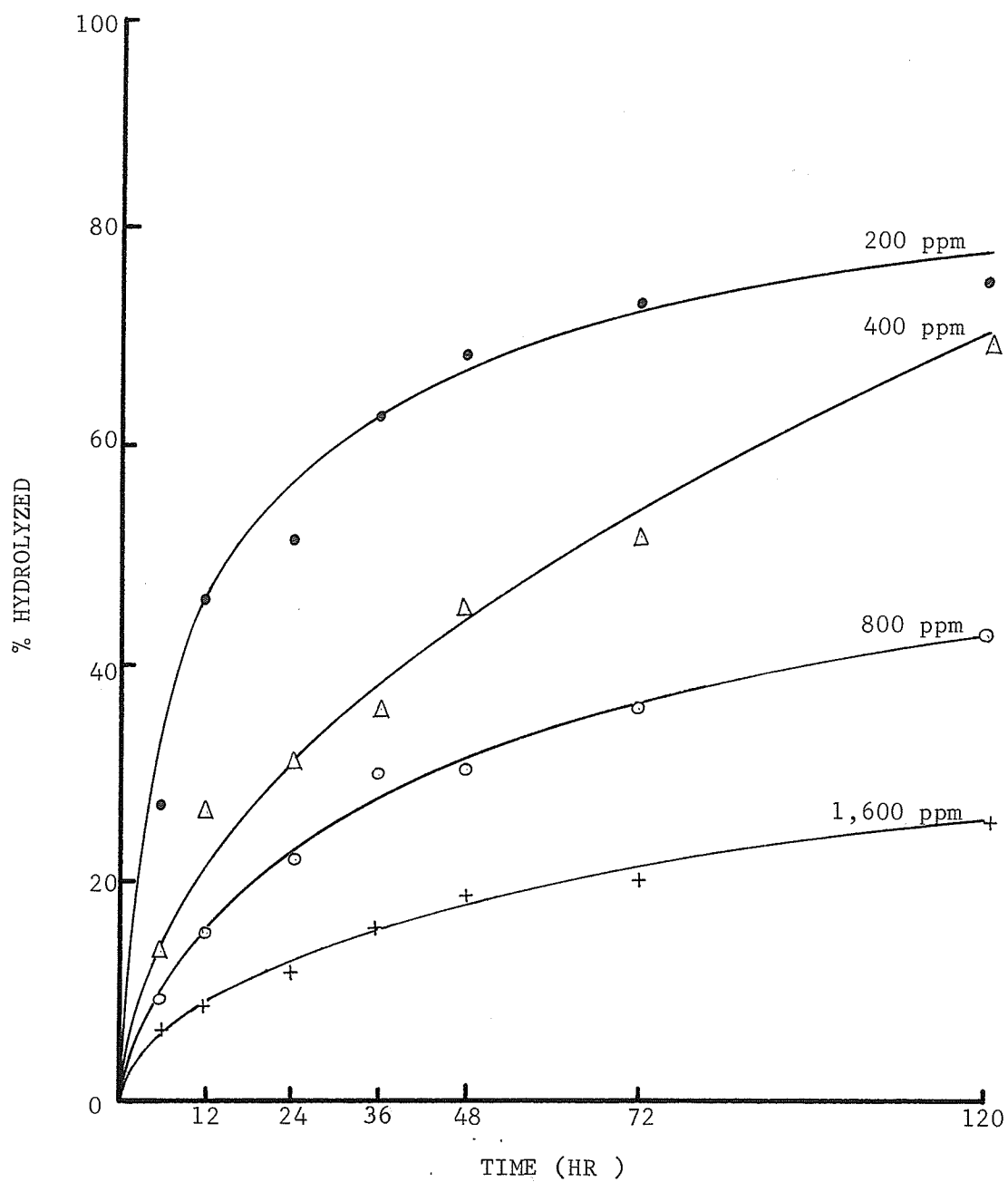
Figure 19. The influence of substrate concentration on the percent hy-
drolysis of sodium tripolyphosphate added to the Lakeland
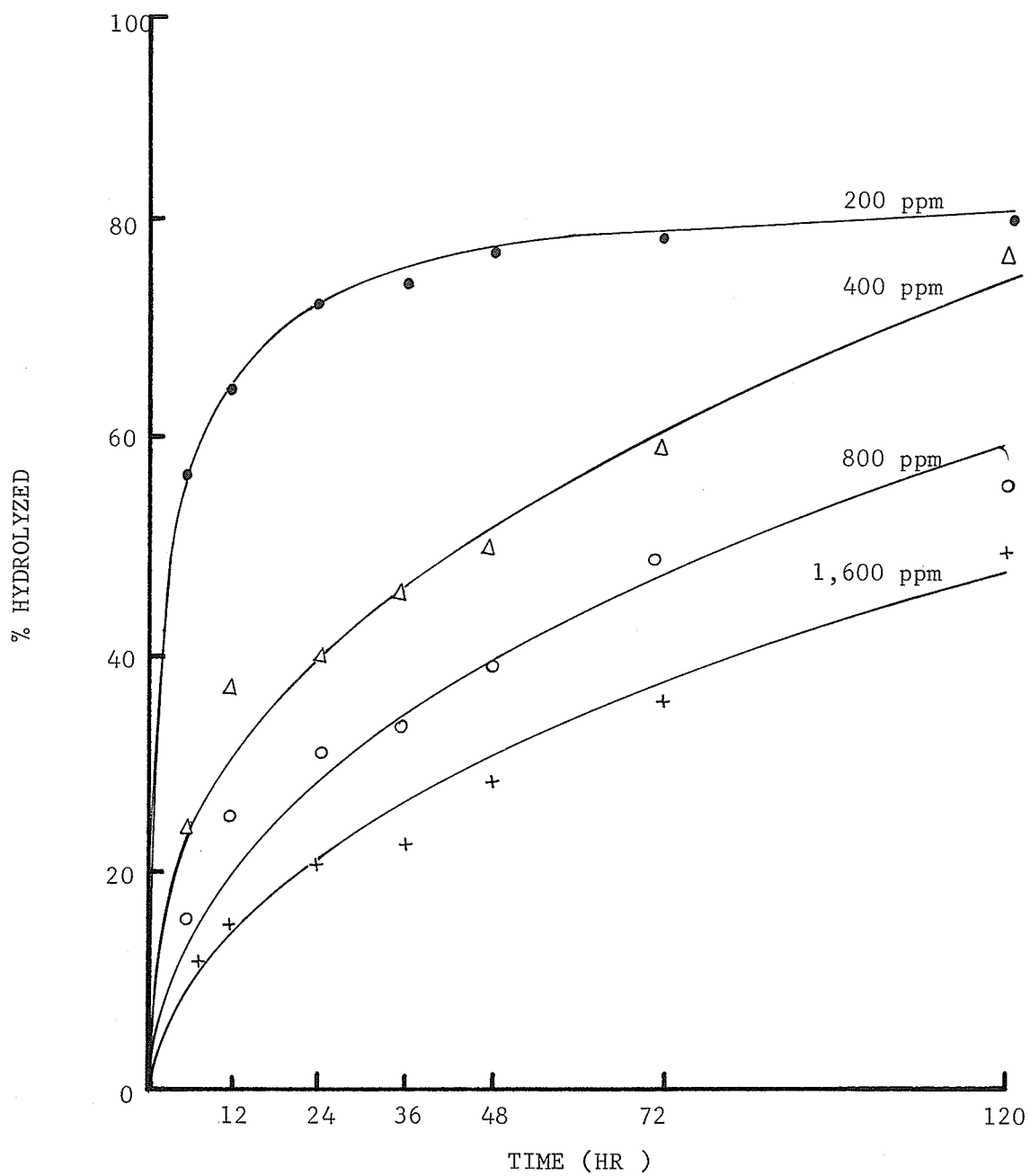soil--water extractable.

Figure 20. The influence of substrate concentration on the percent hy-
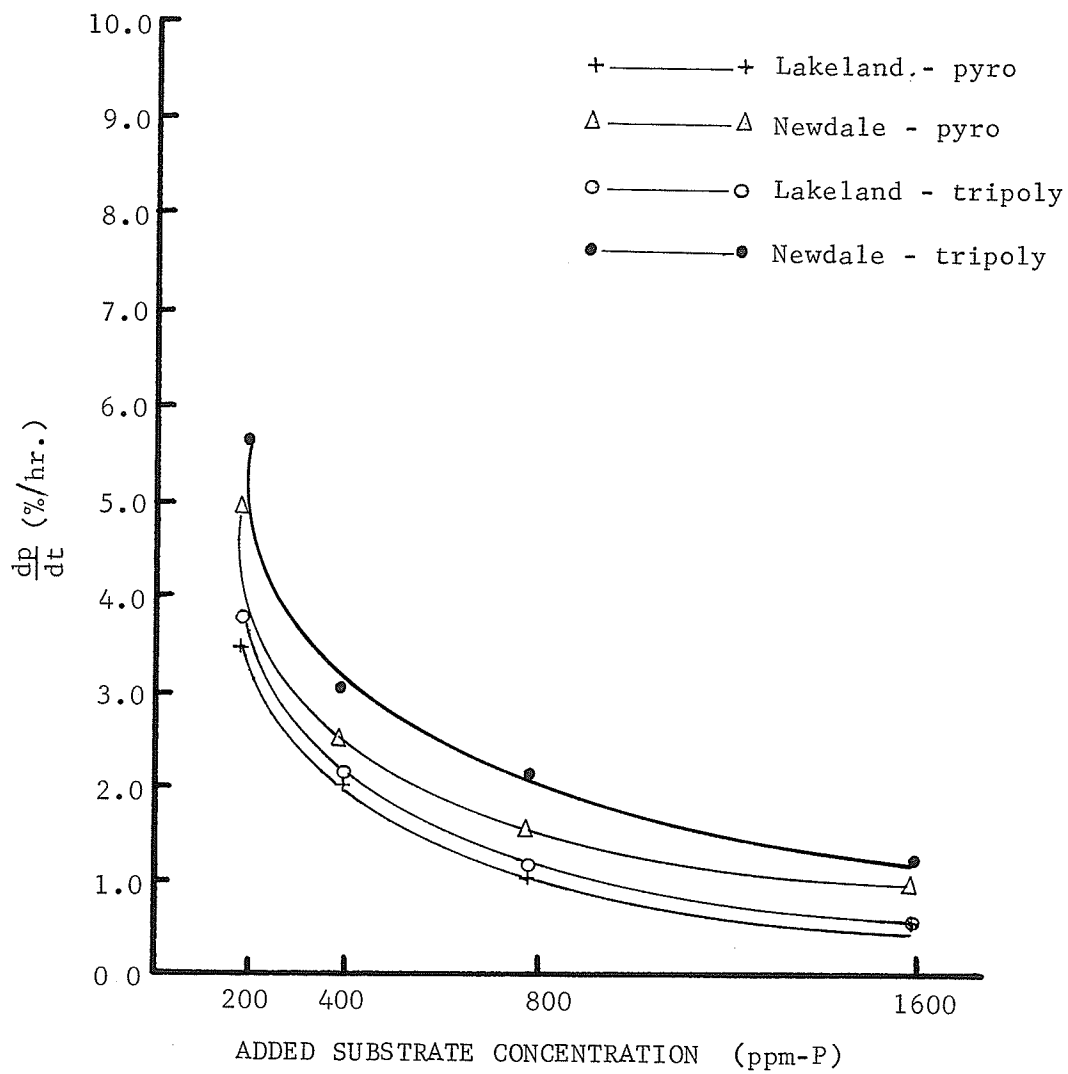drolysis of sodium tripolyphosphate added to the Newdale
soil--water extractable.

Figure 21. Effect of substrate concentration on rate of hydrolysis (% hydrolysis) of sodium pyrophosphate and sodium tripolyphosphate in soil.
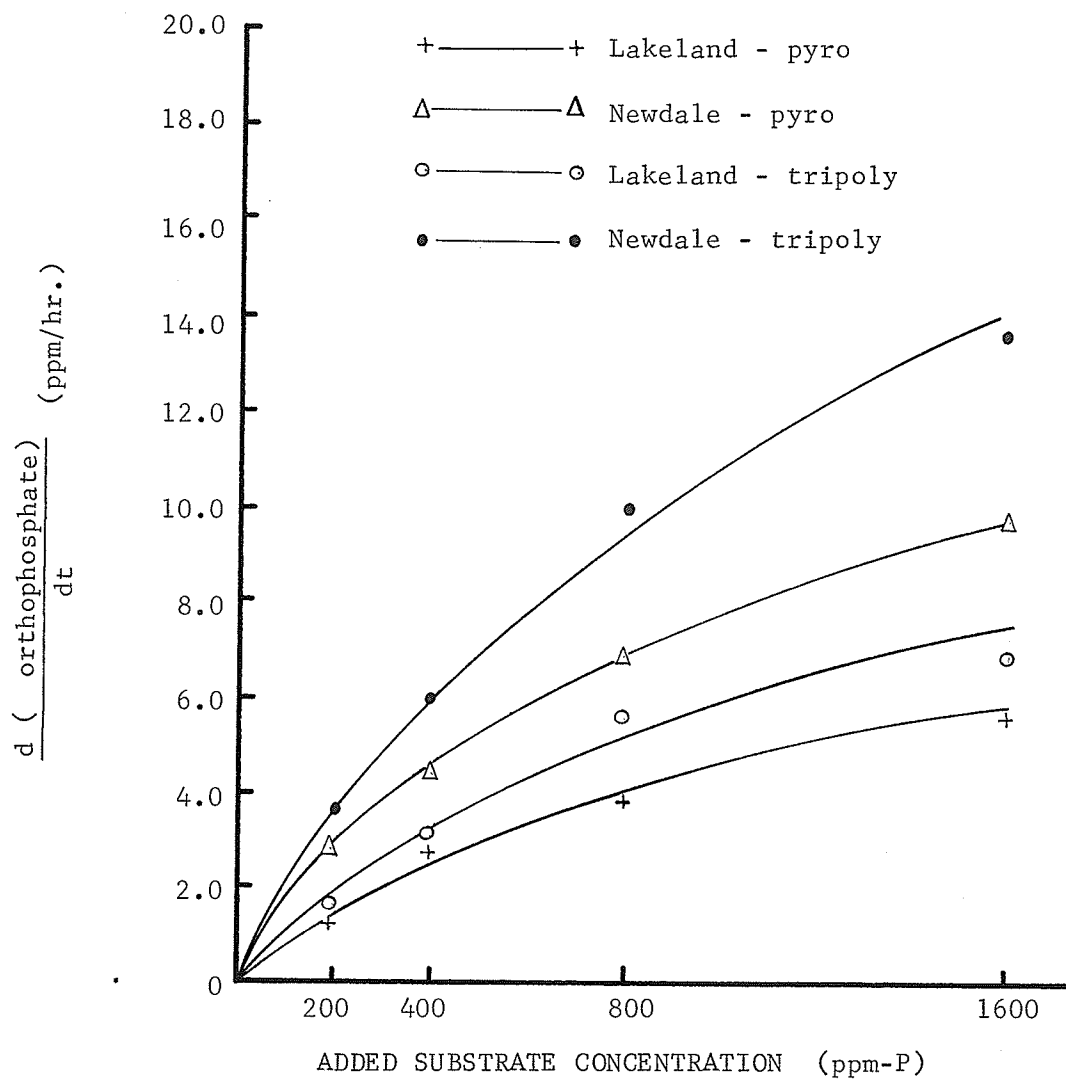
Figure 22. Effect of substrate concentration on rate of hydrolysis of sodium pyrophosphate and sodium tripolyphosphate in soil (orthophosphate produced in ppm-P/hour).

orthophosphate produced increased with increases in the amount of phosphate added to the soils. It is apparent from Figure 22, that higher additions of phosphates would have resulted in still greater rates of orthophosphate produced in both soils.

In the previous study (in A) it was shown that the rate of pyrophosphate or tripolyphosphate hydrolysis in sterile distilled water followed first-order reaction kinetics. Also the degradation of pyrophosphate in soil was assumed to follow first-order reaction kinetics (Experiment 1, equation 22). However, the results from Experiment 3 indicated that the rate equation as expressed by equation (22) was only an approximation. It seems very probable that the rate equation may be similar to that of a Michaelis-Menten type since the rate was dependent upon the initial pyrophosphate or tripolyphosphate concentration (Figure 21).

V   CONCLUSION AND SUMMARY

In recent years, condensed phosphates have been incorporated into some phosphatic fertilizers used in Manitoba. Thus, interest has been focused on the hydrolysis of condensed phosphates as other workers have shown that these condensed phosphates have to undergo hydrolysis prior to utilization by the plant. In an attempt to gain more information on condensed phosphate hydrolysis, several studies were conducted.

A study was conducted to determine the influence of incubation temperature and pH on the rate of hydrolysis of sodium pyrophosphate and sodium tripolyphosphate in sterile distilled water. The rate of hydrolysis was found to significantly increase with increasing temperature. There was little or no hydrolysis observed at $5^{\circ}C$. The rate of hydrolysis increased slightly as the pH was decreased from 8 to 4 for both sodium pyrophosphate and sodium tripolyphosphate solutions. The rate of hydrolysis of sodium pyrophosphate and sodium tripolyphosphate in sterile distilled water was found to be very slow and follow first-order reaction kinetics.

Other studies were conducted to determine the influence of incubation temperature and concentration of substrate on the hydrolysis of sodium pyrophosphate and sodium tripolyphosphate in a Lakeland and Newdale soil. Due to the low recovery of applied pyrophosphate and tripolyphosphate and the fixation of the applied phosphates by the soils, the rates of hydrolysis were expressed as a percentage (the concentration of water or acid extractable orthophosphate/the concentration of total water or acid extractable phosphorus X 100). The rates of hydrolysis of both pyrophosphate and tripolyphosphate increased linearily with temperature at temperatures of 5 to $50^{\circ}C$. Above $50^{\circ}C$, variable results

were obtained. Increases in incubation temperature not only increased the rate of hydrolysis but also increased the total amount of added phosphate hydrolyzed at the end of the incubation period. The Newdale soil hydrolyzed the condensed phosphates at a greater rate than did the Lakeland soil. Also, tripolyphosphate usually hydrolyzed at a greater rate than did pyrophosphate in soils. Phosphorus extracted by water was found to hydrolyze at a greater rate than phosphorus extracted by 0.5N H_2SO_4 . This indicated that strongly adsorbed or precipitated pyrophosphate or tripolyphosphate hydrolyzed at a slower rate than did phosphorus not or only weakly fixed by the soil.

The rate of hydrolysis (% hydrolysis) decreased with increases in the amount of applied pyrophosphate or tripolyphosphate. However, the rate of orthophosphate produced increased with increases in the amounts of substrate added. It was found that the optimum rate of orthophosphate produced would occur at substrate concentrations greater than 1,600 ppm-P.

It was found hydrolysis of pyrophosphate followed first-order reaction kinetics when 200 ppm-P pyrophosphate was added. However, the rate of hydrolysis was found to be dependent upon the initial concentration of pyrophosphate or tripolyphosphate added to the soil. Thus, the hydrolysis of these phosphates in soil may be similar to that of a Michaelis-Menten type.

The rate of hydrolysis of pyrophosphate and tripolyphosphate in soils was much greater than that in sterile distilled water indicating that the hydrolysis in the soil was catalyzed. Even at $5^{o}C$, relatively large amounts of the phosphates added to soils hydrolyzed. In sterile distilled water no or very little of the added phosphates

hydrolyzed.

The rates of hydrolysis of pyrophosphate or tripolyphosphate found for the soils appear to be great enough to supply plants with their phosphorus needs. However, in instances where the rate of hydrolysis limits phosphorus uptake, the efficiency of condensed phosphates would be lowest when applied to soils maintained at a low temperature.

VI BIBLIOGRAPHY

Abbott, G.A. 1909. The rate of hydration of pyrophosphoric acid in aqueous solution. J. Amer. Chem. Soc. 31: 763-770.

Balareff, D. 1911. Z. anorg. u. allgem. Chem. 68: 266. As reported in Van Wazer, J.R. 1958. Phosphorus and its Compounds-- Vol. 1, Chemistry. pp.457. Interscience Publishers, Inc., New York, U.S.A.

Bamann, E. and Meisenheimer, M. 1938. Ber. dt. Chem. Ges. 71B: 2233-2236. As reported by Clesceri, N.L. and Lee, G.F. 1965. Hydrolysis of condensed phosphates--I: Non-sterile environment. J. Air Wat. Poll. 9: 723-742.

Bamann, E. and Meisenheimer, M. 1958. Ber. dt. Chem. Ges. 71B: 2086- 2089. As reported by Clesceri, N.L. and Lee, G.F. 1965. Hydrolysis of condensed phosphates--I: Non-sterile envir- onment. J. Air Wat. Poll. 9: 723-742.

Bell, R.N. 1947. Hydrolysis of dehydrated sodium phosphates. Ind. Engng. Chem. 39: 136-140.

Bell, R.N. 1952. Preparation of sodium tetrametaphosphate. Ind. Engng. Chem. 44: 568-572.

Blancher, R.W. and Hossner, L.R. 1969a. Hydrolysis and sorption reactions of orthophosphate, pyrophosphate, tripolyphosphate and trimetaphosphate anions added to an Elliott soil. Soil Sci. Soc. Amer. Proc. 33: 141-144.

Blancher, R.W. and Hossner, L.R. 1969b. Hydrolysis and sorption of ortho-, pyro-, tripoly-, and trimetaphosphate in 32 midwestern soils. Soil Sci. Soc. Amer. Proc. 33: 622-625.

Brovkina, I.A. 1953. J. Gen. Chem. (U.S.S.R.) 22. As reported by McCullough, J.F., Van Wazer, J.R. and Griffith, E.D. 1956. Structures and properties of the condensed phosphates--XI: Hydrolytic degradation of Graham's salt. J. Amer. Chem. Soc. 78: 4528-4533.

Campbell, D.O. and Kilpatrick, M.L. 1954. A kinetic study of the hydro- lysis of pyrophosphates. J. Amer. Chem. Soc. 76: 893-901.

Clesceri, N.L. and Lee, G.F. 1965. Hydrolysis of condensed phosphates-- I: Non-sterile environment. J. Air Wat. Poll. 9: 723-742.

Crowther, J.P. and Westman, A.E.R. 1954. The hydrolysis of the condensed phosphates--I: Sodium pyrophosphate and sodium tripolyphosphate. Can. J. Chem. 32: 42-48.

Eppley, R.A. 1962. Hydrolysis of polyphosphates by Porphyra and other seaweeds. Physiologia Pl. 15: 246-251.

Forti, G. 1961. Partial purification of a pyrophosphatase from spinach leaves. Biochem. Biophys. Acta. 48: 200-202.

Freiss, S.L. 1952. The energetics of acid-catalyzed hydrolysis of triphosphoric and pyrophosphoric acids. J. Amer. Chem. Soc. 74: 4027-4029.

Gilliam, J.W. 1970. Hydrolysis and uptake of pyrophosphate by plant roots. Soil Sci. Soc. Amer. Proc. 34: 83-86.

Gilliam, J.W. and Sample, E.C. 1968. Hydrolysis of pyrophosphate in soils: pH and biological effects. Soil Sci. 106: 352-357.

Giran, H. 1903. Ann. chim. phys. (VII), 30: 203 as reported in Van Wazer, J.R., Griffith, E.J. and McCullough. 1955. Structure and properties of the condensed phosphates--VII: Hydrolytic degradation of pyro- and tripolyphosphate. J. Amer. Chem. Soc. 77: 287-291.

Green, J. 1950. Reversion of molecularly dehydration sodium phosphates. Ind. Engng. Chem. 42: 1542-1546.

Griffith, E.J. 1959. A nomograph for the hydrolytic degradation of pyro- and tripolyphosphates. Ind. Engng. Chem. 51: 240.

Gunary, D. 1966. Pyrophosphate in soils; some physiochemical aspects. Nature 210: 1297-1298.

Harrow, B. and Mazur, A. 1958. Textbook of Biochemistry. 557 pp. W.B. Saunders, Co., Philadelphia, U.S.A.

Hashimoto, I., Hughes, J.D. and Philen, O.D. Jr. 1969. Reactions of triammonium pyrophosphate with soils and soil minerals. Soil Sci. Soc. Amer. Proc. 33: 401-405.

Hossner, L.R. and Melton, J.R. 1970. Pyrophosphate hydrolysis of ammonium, calcium, and calcium ammonium pyrophosphates in selected Texas soils. Soil Sci. Soc. Amer. Proc. 34: 801-805.

Huffman, E.O. and Fleming, J.D. 1960. Calcium polyphosphate-- Rate and mechanism of its hydrolytic degradation. J. Phys. Chem. 64: 240-244.

Ingelman, B. and Malmgren, H. 1945. Acta. Chem. Scand. 1: 422. As reported in Van Wazer, J.R. 1958. Phosphorus and its Compounds--Vol. I, Chemistry. pp.456. Interscience Publishers, Inc., New York, U.S.A.

Ito, E., Kondo, D. and Watanabe, S. 1955. Studies on sweet potato phosphatase--I: Specificity. J. Biochem. Tokyo 42: 793-803.

Jackman, R.H. and Black, C.A. 1952. Phytase activity in soils. Soil Sci. 73: 117-125.

Karbe, K. and Jander, G. 1942. Kolloid-Beih. 54: 1. As reported in Van Wazer, J.R. 1958. Phosphorus and its Compounds-- Vol. 1, Chemistry. pp.456. Interscience Publishers, Inc., New York, U.S.A.

Karl-Kroupa, E., Callis, C.F. and Seifter, E. 1957. Stability of condensed phosphates in very dilute solution. Ind. Engng. Chem. 49: 2061-2062.

Laidler, K.J. 1965. Chemical Kinetics. pp.219-220. McGraw-Hill Publishers, Inc., New York.

MacIntire, W.H., Hardin, L.J. and Oldham, F.D. 1937. Fertilizers. Ind. Engng. Chem. 29: 224-234.

McGilrery, J.D. and Crowther, J.P. 1954. The hydrolysis of condensed phosphates--II: (A) The role of the hydrogen ion in the hydrolysis of sodium pyrophosphate; (B) The dissociation constants of pyrophosphoric acid. Can. J. Chem. 32: 147-185.

Morgan, R.A. and Swoope, R.L. 1943. The useful life of pyro-, meta- and tetraphosphates. Ind. Engng. Chem. 35: 821-824.

Mozersky, S.M., Pettinati, J.D. and Kolman, S.D. 1966. An improved method for the determination of orthophosphate suitable for assay of adenosine triphosphatase activity. Anal. Chem. 38: 1182-1187.

Newmark, M.Z. and Wenger, B.S. 1960. Preparation and some properties of an acid phosphatase from wheat seedlings. Archs. Biochem. Biophys. 89: 110-117.

Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Dept. Agr. Circ. 939.

Pfanstiel, R. and Iler, R.K. 1952. Potassium metaphosphate, molecular weight, viscosity behavior and rate of hydrolysis of non-cross-linked polymer. J. Amer. Chem. Soc. 74: 6059-6064.

Philen, O.D. Jr. and Lehr, J.R. 1967. Reactions of ammonium polyphosphates with soil minerals. Soil Sci. Soc. Amer. Proc. 31: 196-199.

Ridley, A.O. 1958. Effect of mineral fertilizers and manure on the phosphorus content of a clay soil and on crop yields. M.Sc. Thesis, Univ. of Manitoba, Winnipeg, Man.

Roche, J. 1950. Phosphatases. Vol. 1, Pt. 1--The Enzymes. pp. 473-510. (J.B. Sumner and K. Myrback, Eds.) Academic Press, Inc., New York.

Savant, N.K. and Racz, G.J. 1971. Hydrolysis of sodium pyrophosphate and tripolyphosphate by plant roots. Soil Sci. (In press.)

Savant, N.K. and Racz, G.J. 1971a. Unpublished data, Dept. of Soil Science, Univ. of Manitoba, Winnipeg, Man.

Scott, C.O. 1958. Sorption of orthophosphate and non-orthophosphate phosphorus by soils. Ph.D. Thesis. Iowa State College, Ames, Iowa.

Smith, M.J. 1959. Independence in hydrolysis of condensed phosphates. Can. J. Chem. 37: 1115-1116.

Striplin, M.M., McKnight, D. and Megar, G.H. 1958. Phosphoric acid of high concentration. J. Agr. Food Chem. 6: 298-303.

Sutton, C.D., Gunary, D. and Larsen, S. 1966. Pyrophosphate as a source of phosphorus for plants: II. Soil Sci. 101: 199-204.

Sutton, C.D. and Larsen, S. 1964. Pyrophosphate as a source of phosphorus for plants. Soil Sci. 97: 196-201.

Takefuji, K. 1966. Properties of potassium polyphosphate as fertilizer. Sci. Soil Manure, Tokyo, 38: 137-178.

Thorup, R.M. 1957. Retention, release and hydrolysis of potassium metaphosphate in soils. M.S. Thesis, N.C. State Univ., Raleigh, North Carolina, U.S.A.

Thorup, R.M. and Mehlich, A. 1961. Retention of potassium meta- and ortho-phosphates by soils and minerals. Soil Sci. 81: 38-43.

Van Wazer, J.R. 1958. Phosphorus and its compounds--Vol. 1, Chemistry. 2046 pp. Interscience Publishers, Inc., New York, U.S.A.

Van Wazer, J.R., Griffith, E.J. and McCullough, J.R. 1952. Hydrolysis of condensed phosphates. J. Amer. Chem. Soc. 74: 4977.

Van Wazer, J.R., Griffith, E.J. and McCullough, J.F. 1954. Analysis of phosphorus compounds. Anal. Chem. 26: 1755-1759.

Van Wazer, J.R., Griffith, E.J. and McCullough, J.F. 1955. Structure and properties of the condensed phosphates--VII: Hydrolytic degradation of pyro- and tri-polyphosphate. J. Amer. Chem. Soc. 77: 287-291.

Walkley, A. and Black, T.A. 1934. An examination of the Degljareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37: 29-38.