A LABORATORY STUDY OF THE BEHAVIOUR OF ADDED PHOSPHATE IN AN ALLOPHANIC SOIL

by

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I. A. Introduction.

A feature in the maintenance of high producing pastures, on the yellow-brown loam soils in Taranaki, is the need for high annual rates of phosphatic fertilizer application.

Burgess and Davies (I95I) in some early investigations into soil fertility problems in Taranaki noted that the yellow-brown loams could apparently accumulate large amounts of phosphate in a form which was difficult for plants to extract. Consequently to ensure adequate plant phosphate nutrition large annual dressings of superphosphate are recommended for high producing pastures on these soils.

This high phosphate-retaining property has been attributed to the presence of active aluminium supplied by allophane, the predominant clay mineral in these soils. (Saunders I959 (b)).

The only recent intensive study to be made on phosphate relationships in the yellow-brown loams is that of Saunders (I959 (a)(b)(c)) who carried out chemical fractionation of the phosphate in New Plymouth black loam, a soil which may be considered as representative of the group as a whole. His sampling sites were on areas of different topdressing histories, and although he was able to draw certain conclusions in respect of the trends of phosphate fixation this approach does not provide any information on the shorter term fate of applied phosphate. Information of this kind is, however essential if a rational explanation of the established need for substantial annual additions of phosphate to these soils, is to be forthcoming.

The present study was undertaken with the object of tracing, in the laboratory, the course of the reaction of phosphate with New Plymouth black loam over approximately that period which would normally elapse between successive topdressing operations in the field both in respect of the forms of phosphate binding and the solubility and exchangeability ofthe reaction products. It was of interest also to determine whether the trends of fixation reported by Saunders (I959 (a)(b)(c)) in the field would be reproduced under laboratory conditions.

* * *

B. Literature Review.

The literature relevant to the present study may be grouped as follows:-

I. The effects of time, and other factors, as influences on -

   (a) the forms of phosphate binding in soils
   (b) the solubility and exchangeability of fixed phosphate

2. The determination of isotopically exchangeable phosphate in soils.

I.(a) The forms of phosphate binding in soils.

Several workers have reported that the main fraction of soil responsible for the fixation of added phosphate is the clay component (Moschler et al I957, Fine and Bartholomew (I946).

The mechanisms for the reaction of soluble phosphate with soil constituents, and the identification of the products formed have been well documented (Wild I950, Haseman et al I950, Wright and Peech I960, Ellis and Truog I955, Coleman et al I960, Hemwall I957, Kittrick and Jackson I956, Lindsay et al I962, and Cho and Caldwell I959). However most of these reports have been made after using model systems in the laboratory. Identification of the various reaction products possible in soil systems is much more difficult and very often the evidence is only circumstantial.

In general it is agreed that calcium,iron and aluminium are the main elemental constituents of soils which are responsible for retention of phosphate. (Wild I950, Dean I949).

Fractionation techniques have been devised to categorise the inorganic fraction of soil phosphorus into three major forms - aluminium-bound, iron-bound and calcium-bound. (Dean I938, Ghani I943, Chang and Jackson I957 (I), Khin and Leeper I960, Fife I959). These techniques have enabled precise studies to be made on changes in phosphate binding in soils under a varying range of conditions.

The use of phosphate fractionation procedures for tracing soil/phosphate reactions has been criticised by several workers. Yuan, Robertson and Neller (I960) claim that chemical fractionation procedures give a low percentage recovery of the total phosphate applied to soils, whereas higher percentage recoveries can be obtained when an isotopic tracer method is employed.

Volk and McLean (I963), who do not support the findings of Yuan et al, were able to recover most of the phosphate, applied to soils, as the iron - bound and aluminium - bound fractions. Laverty and McLean (I96I) criticised the use of the fractionation procedure for studying soil phosphate binding, because it takes no account of the differences in solubilities between freshly formed phosphates and the more aged crystalline compounds. However they concluded that the method may be used empirically until such time as a better method emerges to enable studies in trends in phosphate fixation by soils.

One important factor governing the role of calcium, iron, and aluminium in the phosphate fixation process appears to be the pH of the system. Under neutral and slightly alkaline conditions, calcium and magnesium are probably responsible for phosphate retention, while under slightly acid conditions, manganese or aluminium may be more important in maintaining a low phosphate concentration in the soil solution. Iron would appear to be important under very acid conditions. (Teakle I928). Chiang (I963) showed that under waterlogging conditions in acid soils, the amount of the different forms of fixed phosphate was Fe - bound P > Al - bound P > Ca - bound P. However in alkaline soils the order was Ca - bound P > Fe - bound P > Al - bound P. When the pH of acid soils was raised, following the addition of organic matter, the amounts of Ca - bound P and Fe - bound P increased and Al - bound P decreased. In the alkaline soils, Ca - bound P and Al - bound P increased and Fe - bound P decreased after organic matter additions.

Saunders (I959 (a)) summarised the literature on the effects of pH on phosphate retention:-

(i) In iron and aluminium systems the phosphate in solution is at a minimum between about pH 2 and pH 7.

(ii) In calcium systems, the phosphate in solution is at its minimum above about pH 6.5.

(iii) The pH of minimum solubility in iron systems is about one pH unit lower than in aluminium systems.

Rathje (I960) presented a diagrammatic representation of the situation that could exist in the soil:-

$$\text{Ca - P} + \begin{matrix}\text{Al}\\\text{Fe}\end{matrix}\ \text{hydroxide} \rightleftharpoons \begin{matrix}\text{Al}\\\text{Fe}\end{matrix}\ \text{"hydroxide phosphate"} + \text{CaOH}$$

He suggested that the equilibrium was shifted to the left by an alkaline reaction and to the right by an acid reaction. However this is not the complete picture because some calcium-bound forms of phosphate can be formed under acid conditions.

Dean (I938) showed that much of the phosphate in the neutral soil appeared to be present as tri - calcium phosphate or apatite, and that in acid soils it was predominantly the iron -, aluminium- or mono - and di -, calcium -, phosphate forms.

Martin and Laudelout (I959), Ceccoui (I960) and Cho and Caldwell (I959) discussed the effects of pH on the form of phosphate retained by soils. However it is unlikely that pH alone is the only factor determining the distribution of applied phosphate between the calcium, iron and aluminium bound fractions in soil systems.

The amount of phosphate added may determine the form and quantity in which phosphate is fixed in soil. (Fiskell and Spencer I964)(Abdel-Samie, Hamdi and Barrada I962). Fiskell and Spencer (I964) showed that when a soil, whose native phosphate was largely in the form of iron - bound phosphate, was fertilised with

800 lbs phosphate/acre, most of the phosphate added accumulated as aluminium - bound phosphate with lesser amounts of the calcium - bound and iron - bound forms. However when the addition of phosphate was increased to I2,800 lbs phosphate/acre, there was considerably greater formation of iron - bound, and calcium - bound forms although the aluminium - bound form still showed the greatest increase.

Another important factor likely to affect the form in which phosphate is "fixed" in soils, appears to be the total fixation capacity of the soil. Laverty and McLean (I96I) showed that soils of low fixation capacity fixed 2½ times as much phosphate in the aluminium-bound fraction as in the iron - bound form, while the reverse was true for soils of high fixation capacity.

Volk and McLean (I963) showed a tendency to recover more than half of the applied phosphate as iron - bound phosphate in soils of high phosphate fixation capacity, and more than half as aluminium - bound phosphate in those soils of low phosphate fixation capacity.

Kaila (I963) showed that intensive phosphate fertilising led to accumulation of phosphate bound to iron and aluminium in the arable layer.

The above reports on the rates of phosphate fertilization and the fixation capacities of soils can be reconciled when it is remembered that the magnitude of the fixation process will depend largely on the mineral composition of the soil. Therefore a consideration of the total phosphate fixation capacity of a soil does not always allow predictions as to the form in which phosphate will be bound in that soil. It is generally accepted that where abundant aluminium is present as a soil constituent the initial binding of added phosphate is likely to be in an aluminium-bound form. However a soil of high total fixation capacity but with a small percentage of aluminium sites available for fixation could conceivably bind phosphate in a form other than aluminium-bound phosphate. e.g. iron - bound P, or calcium - bound P.

Heck (I934) showed that if the ratio of active calcium to active aluminium and iron is high, the fixation of phosphate is largely in the calcium - bound form. If the reverse is true then iron - bound and aluminium - bound forms of fixed phosphate result.

It has been suggested that although the aluminium component of soils may be largely responsible for the magnitude of the phosphate sorption by soils, iron may be important in determining the strength of retention of the phosphate (Williams, Scott and McDonald I958).

Two types of phosphate adsorption were observed in volcanic ash soils by Onikura (I963) - one type had a soil phosphate system of low initial phosphorus concentration and the phosphate solid phase tended towards basic aluminium phosphate, while the other form had high initial phosphorus concentration and the solid phase was neutral and/or acidic aluminium phosphate.

Burgess and Davies (I95I) and Saunders (I959 (b)) found no increase in organic phosphorus in yellow - brown loams as a consequence of phosphate topdressing. In contrast, Jackman (I95I) on a yellow - brown pumice soil found that added phosphate accumulated largely in the soil in an organic form. Jackman produced evidence that no build up in organic phosphorus occurred if a virgin pumice soil was topdressed and left fallow for five months. However, where clover and grass were grown, about I7% of the applied phosphate was recovered in the organic form in the soil.

Williams (I950) found that on a red - brown earth the application of superphosphate caused no increase in organic phosphorus content of the soil when the cover crop was a cereal. If the cover crop was pasture, the application of superphosphate fertilizer caused an increase in the organic phosphorus fraction of the soil phosphorus.

The lack of a universal method for the estimation of the organic fraction of soil phosphate could account for the anomalous results reported for changes in organic phosphorus following phosphate fertilization.

However some workers appear to neglect the organic fraction of soil phosphate during investigations into the fate of applied phosphate to soil. Fiskell and Spencer (I964) found that phosphate added to soil could not be accounted for in the calcium - bound, iron - bound and aluminium - bound forms of soil phosphate. They postulated the formation of occluded aluminium phosphate. Chang and Chu (I96I) did not take into account organic phosphorus when considering changes in soil phosphate following fertilizer application.

Several workers have demonstrated that under conditions of prolonged alternate wetting and drying of soil, there appeared a tendency for a reduction in the percentage of phosphate in the aluminium - bound fraction and an increase inthe percentage distribution of the iron - bound forms. (Mack and Barber I960) (Yuan, Robert and Neller I960) (Yuan I960) (Hsu I964) and (Chang and Chu I96I).

Mandal (1964) working with soils which were kept under waterlogged conditions in test - tubes showed that with time the control soils showed a slight increase in acetic - acid - extractable phosphate, a slight decrease in ferric - bound phosphate, but no change in either aluminium - bound or calcium - bound forms of phosphate.

In surface soils with an average phosphorus content of 675 ± 26 p.p.m. Gotoh et al (1963) showed the total phosphorus changed only slightly with increasing time. However they did show definite trends - organic phosphate (which represented 10.5% of the total phosphorus in the soil) increased gradually with time, aluminium - bound phosphate (5.9% of total) increased, as did iron - bound phosphate (19% of total). However calcium - bound phosphate (21.6% of total) decreased with time. In the soils used in this work, highly insoluble phosphate (43.5% of total) was the dominant form of fixed phosphate.

The apparent pK_{SP} value for aluminium - phosphate ranges from 28 - 32, and that for iron - phosphate from 33 - 35, indicating a much lower solubility of the latter. (Chang and Jackson 1957 (2)).

An explanation for the observed changes in distribution between the 3 categories of phosphate most commonly considered in these experiments, viz calcium - bound, aluminium - bound and iron - bound phosphate is offered by Chang and Chu (1961):- "initially the reaction of added soluble phosphate occurs on the surfaces of the solid phases with which it comes in contact in the soil. Therefore it is the specific surface area of the solid phases associated with the iron, calcium and aluminium irons, that determines the form and amount of the phosphate formed. Since the main site of phosphate fixation is the clay fraction of the soil, and since the clay content of aluminium is higher than iron or calcium, the most likely initial form of phosphate is aluminium - bound phosphate. However, because the solubility product of iron - bound phosphate is lower than that of either aluminium - bound or calcium - bound phosphate, it is probable that as time progresses the aluminium - bound, and calcium - bound forms of phosphate in the soil will change to iron - bound forms."

In yellow - brown loams, where long and continued applications of phosphatic fertilizer have been practised, most of the increase in phosphate can be accounted for in the aluminium - bound fraction (Saunders I959(b)). Calcium binding of phosphate appears to play no part in the fixation of added phosphate in these yellow - brown loams.

Of particular importance in the study of phosphate fixation in these soils is the very high specific area of the clay particles, some 400 m^2/g (Birrell and Fieldes I952). This large surface area allows a high proportion of the aluminium to be present at the colloid surface where it is available for fixing phosphate. Fieldes and Swindale (I954) showed that the clay fraction of the yellow - brown loams had a C.E.C. of 80 - I00 meq/I00g. (Saunders (I956) quoted 30 - 50 m.e.%) and could fix I00 m.mol P_2O_5/I00g, in a form insoluble in I% citric acid. Most of the phosphate fixation capacity of these soils can be attributed to their allophanic content and various mechanisms for the actual fixation process have been postulated. Fieldes and Schofield (I960) suggested that phosphate was held in a difficultly available form at tetrahedral aluminium sites similar to those found in montmorillonite and micaceous clay minerals, where aluminium substitutes extensively for silicon in silica tetrahedra. Saunders (I959 (b)) in a study on yellow - brown loams, which have a particularly high allophane content, showed that this soil had a capacity to retain large amounts of phosphate in an insoluble form. Wada (I959) showed that allophane was rapidly converted to the insoluble phosphate mineral "taranakite" following phosphate treatment of the soil with ammonium phosphate solution at pH4. On the yellow - brown loams (Saunders I959(c)) the retention of phosphate was at a maximum between pH 3.5 and pH 7.0. Wada (I959) showed the formation of taranakite from allophane proceeded faster at pH 4 than at pH 7.

Birrell (I96I) showed that by treating allophane with soluble ammonium phosphate,about 25% of the silica content of the soil was dissolved, but he concluded that the main reaction liable to occur was the solution of alumina followed by the precipitation of taranakite. This agreed with the mechanism proposed by Kittrick and Jackson (I956) for the retention of phosphate by kaolin.

Due to its extremely fine particle size and behaviour as a slowly reversible gel of high water content, allophane appreciably affects the physical properties of the clays in which it occurs(Gradwell and Birrell I954, Birrell and Fieldes I952).

According to the work of Chang and Jackson (I957 (2)) and Chang and Chu (I96I) it would be expected that given time phosphate initially bound as aluminium - bound phosphate in allophanic soils would be gradually converted into iron - bound phosphate.

(b) The solubility and exchangeability of fixed phosphate.

When soluble phosphate is added to a soil, the form in which it becomes initially bound is often prone to further changes. Many instances are reported where conspicuous changes occur between the forms of bound phosphate in soils as a result of environmental conditions, or the effects of time.

Wild (1950) noted that when soluble phosphate is added to a soil, its solubility in a variety of acids decreased with time.

Other workers have recorded similar changes in solubility, and rate of fixation, of added fertilizer phosphate.

Cecconi (1960) showed that the first products of phosphate fixation are often amorphous and possess a high ability to exchange the phosphate component. This capacity to exchange progressively decreases with the loss of amorphous characteristics during aging.

Benko, Hampl and Vnuk (1963) demonstrated that the fixation of added phosphate increases, and the proportion of water - soluble phosphate decreases, with increasing time of contact of the phosphate with the soil.

Smith and Simpson (1950) were able to show that, in general, the amount of phosphate soluble in water decreases rapidly with time, following phosphate additions over a variety of soil types.

Sell and Olson (1946) showed that available phosphate (i.e. soluble in 0.7N HCl + 0.3% ammonium molybdate) in a grazed pasture decreases with time when the phosphate was topdressed or incorporated into the soil. The depletion was greatest with the highest rate of phosphate application. However, Sell and Olsen could not decide whether the loss of valuable phosphate was due to removal by plant and animal, or to soil fixation.

Sillampaa (I96I) concluded that fixation of fertilizer phosphate was rapid immediately following its application to the soil, but progressively slowed down so as to exhibit a " double logarithmic function of time."

Chiang (I963) showed that when soils, both acidic and alkaline, were waterlogged, the amount of soluble phosphate increased as a result of hydrolysis of the difficultly-soluble aluminium-bound, and iron-bound, phosphates which were present before waterlogging.

There are few reports in the literature concerning the fraction of phosphate,added to soils, that remains in an exchangeable form for any length of time. This has probably been due to the lack of suitable techniques for a careful appraisal of the problem.

Mattingly, Russell and Jephcott (I963) showed that about one-half of the phosphate given in consecutive applications of superphosphate to the soil remained isotopically exchangeable, and about one-third still exchanged after phosphate additions had ceased for two years.

2. The determination of isotopically exchangeable phosphate in soils.

A radioactive isotope of phosphorus (^{32}P) is used in soil systems for the estimation of the isotopically exchangeable fraction of soil phosphate. During this determination, phosphorus of high specific activity may be added to an equilibrated soil / solution system without markedly disturbing the equilibrium state.

It is probable that plants draw on the isotopically exchangeable fraction of soil phosphate for the major proportion of their phosphorus requirement. An accurate method for the determination of isotopically exchangeable phosphate in soils, together with a knowledge of factors which influence its magnitude in soils, may become necessary information for the prediction or assessment of the "phosphate-supplying power" of soils for plant growth.

A review of methods proposed for the estimation of isotopically exchangeable phosphate in soils now follows;

McAuliffe et al (I948) were pioneers in this field, and they used a method whereby soil was shaken for four days with distilled water. A small amount of carrier-free radioactive ^{32}P-phosphate was then added, and the radioactivity in the soil solution was determined at various intervals.

In I953, Gunnarsson and Fredriksson determined a single value for the labile phosphorus pool in the soil from considerations of the distribution of radioactivity between soil and solution and the total phosphorus in solution, following a fourteen day equilibration of soil with labelled phosphorus.

Talibudeen (I954) extracted the soil, prior to addition of radioactive phosphorus, with dilute ammonium citrate and potassium chloride. He assumed that this would allow a greater ratio of ^{3I}P in solution to ^{3I}P on the soil, without altering the size of the "labile pool of phosphate".

Barbier et al (I954) obtained values for the labile phosphate in different soils by tagging suspensions of soil in water with carrier - free ^{32}P - phosphate, shaking for a period of 24 hours and analysing the solutions for ^{3I}P - phosphate and ^{32}P - phosphate.

In I954, Scott - Russell presented an equation for calculating the total isotopically exchangeable phosphorus (or the "E value") in a soil:

$$E = \frac{yx^t}{y^t} - x$$

where:

x is the orthophosphate added

y is the ^{32}P - phosphate added

x^t is the orthophosphate in solution at equilibrium

y^t is the ^{32}P - phosphate in solution at equilibrium

Scott - Russell found that in one soil isotopic equilibrium was not reached until 20 days after the addition of radioactive phosphorus to the soil / solution system.

Amer (I962) employed an anion - exchange resin to extract the isotopically exchangeable phosphate from the soil, after the soil had been equilibrated with a solution of orthophosphate and ^{32}P - phosphate. He determined the ^{32}P - phosphate content of the resin and the extract. Where the ^{32}P - phosphate content was greater on the resin than in the soil extract, he concluded that the soil had bound some ^{32}P - phosphate in a form which was no longer exchangeable with the orthophosphate in solution.

In I958, Yuan cast some doubt on the validity of using ^{32}P - phosphate as a tracer of ^{31}P - phosphate in soils. He reported that differential adsorption of the two isotopes of phosphorus, occurred on glass. Yuan showed that the sorption of ^{32}P - phosphate onto glass was greater than that for ^{31}P - phosphate, at a solution temperature of 95°C.

Olsen and Watanabe (I959) and Arriaga e Cunha et al (I960) do not endorse the findings of Yuan as to the different adsorptions of ^{31}P - phosphate and ^{32}P - phosphate.

Olsen and Watanabe (I959) state that, the same chemical reactions occur when ^{32}P and ^{31}P exist in solution in the orthophosphate form, and ^{32}P - phosphate serves as an accurate tracer for ^{31}P - phosphate in soil studies. They suggest that the ^{32}P - phosphate sample used by Yuan may have contained some ionic form of phosphorus other than orthophosphate. This, then, would account for the anomalous behaviour of ^{32}P - phosphate in his experiments.

Arriaga e Cunha et al (I960) did not confirm Yuan's reports while using ion - exchangers which absorbed about 30% of the labelled phosphorus in solution.

From a study of the literature it would appear unlikely that the two isotopes of phosphorus, in the orthophosphate form, would be differentially absorbed by soil systems. Therefore ^{32}P - phosphate appears to be a suitable tracer for the reactions of ^{31}P - phosphate in soils.

In reports on experiments dealing with the isotopically exchangeable fraction of soil phosphorus, it is generally the phosphorus associated with soil surfaces that is measured, and this "surface - phosphorus" fraction is often correlated with the amounts of soil phosphorus available to plants.

A further advance in the study of isotopically exchangeable phosphorus in soils is the consideration of the rates of exchange of phosphorus on soil complexes. This consideration is important for determining the strength of retention of fixed phosphate and, presumably, its availability to plants.

Wiklander (1950) determined the rate at which labelled phosphate absorbed on soils exchanged with phosphate ions in solution and concluded that the exchange was governed by the law of mass action.

In Russia, Fokin (1963) used isotopes to show that during phosphate absorption most of the absorption occurred in the first 5 - 10 minutes and equilibrium was reached after 5 - 7 days. He also noted that phosphate absorption occurred in various stages, but did not define either the rates of absorption or exchange.

Seatz (1954) and Talibudeen (1957) attempted to explain in greater detail the characteristics of the various fractions of isotopically exchangeable phosphorus.

Seatz (1954) shook soil with water for four days before adding radioactive phosphorus. He then measured the radioactivity in solution at various time intervals up to 7 days. From the results he plotted a graph of the fractional activity in the soil against the log of time and suggested that one or two inflexion points could be noted in the resultant curve. He assumed that these portions of the graph were associated with phosphate fractions of the soil which had varying ability to dissociate.

Talibudeen (1957) showed that the determination of total isotopically exchangeable phosphate in the laboratory was affected by a number of factors. Among these factors considered were the soil / solution ratio, the amount of orthophosphate added with the isotope, the equilibrium pH and the relationship between the phosphate extracted, the "surface - phosphate" and the total labile pool of phosphate. He suggested three forms of isotopically exchangeable phosphate ; rapidly - exchanging, slowly - exchanging and very slowly - exchanging phosphates, which were in an equilibrium state in the soil.

A further advance in this field has been the development of a technique to allow clear differentiation of the rates of exchange of exchangeable phosphate in soil systems.

In a paper by Wilson and Furkert (1962) a technique was outlined which enabled a study to be made of the continuous adsorption of water onto solid surfaces. After the addition of tritiated water to a solid / water system continuous counts of the activity in solution were obtained using a Geiger tube, coupled to a strip-chart recorder. A graph of count rate versus time was plotted on a log plot and the resultant curve was mathematically resolved into a number of straight lines. From the intercepts of the lines at the zero time axis, the amounts of each type of water adsorbed onto the clay, could be calculated. Also, from the slopes and intercepts of the lines, the specific rate constants could be calculated for each type of water present.

Shao (1960) used a fully - equilibrated soil / water system to study rates of exchange of added carrier - free ^{32}P - phosphate with soil phosphate. Using a graphical analysis technique, similar to the one applied to the tritiated water experiment of Wilson and Furkert (1962) described above, Shao was able to identify distinctive fractions of the total isotopically exchangeable phosphate of soils.

Other workers have attempted to identify one or two fractions of the exchangeable soil phosphate with the aid of radioactive phosphate tracers.

Machold (I963) used a chemical soil phosphate fractionation procedure after he had added ^{32}P - phosphate to a soil. His results indicated that aluminium - bound and iron - bound forms of phosphate were subject to fast isotopic exchange.

MacKenzie (I962) produced evidence that in most soils the aluminium - bound phosphate fraction of soil phosphate was in equilibrium with the water - soluble phosphate fraction. The indications from studies in 0.0IM $CaCl_2$ solutions were that aluminium - bound phosphate supplies most of the phosphate in the soil solution.

Mazel et al (I963) showed that ^{32}P - phosphate added to a derno - podzolic soil entered the "available - phosphate" fraction, and not the "unavailable - phosphate" fraction, of the soil phosphate. From the evidence available it would appear that eventually some correlation may be obtained between the amount of isotopically exchangeable phosphate in soils and the amount of phosphate available to plants.

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II. Materials and Methods.

A. Materials.

Description of Soils.

The five soil samples of New Plymouth black loam used in this investigation were supplied by Dr. W.M.H. Saunders of the New Zealand Soil Bureau. These samples are representative of five sites, all of which were free - draining and had been under permanent pasture for a minimum of twenty - five years. The field history of the soils is shown in Table I.

The bulk samples of each soil consisted of one - hundred, one - inch diameter, cores which were removed from an area of approximately one - fifth of an acre.

When received, the soils had been air - dried and before use they were screened through a 2 m.m. sieve.

Tables 2 and 3 show relevant data extracted from the report on these soils by Saunders (I959 (b)).

Table 1. Field history of soils.

Nomenclature ascribed to soil	Sampling depth	Annual topdressing rate.	Total period of application	Total phosphate added.
	(inches)	(lbs P/acre)	(years)	(lbs P/acre)
No P	0 - 2	Nil	-	Nil
Mod P (a)	0 - 2	20 - 30	20 - 30	625
Mod P (b)	0 - 2	20 - 30	20 - 30	625
High P (a)	0 - 2	(20 - 30 (60 - 80	(6 (7	(640
High P (b)	0 - 2	60 - 80	7	490

Table 2. Some chemical properties of the soils.

Soils	pH	C.E.C.	T.E.B.	Ex.Ca	Ex.K.	C	N	C/N	C/org.P
		m.e.%	m.e.%	m.e.%	m.e.%	%	%		
No P	5.8	39.9	20.3	15.0	0.75	13.5	1.06	13	97
Mod P (a & b)	5.8	43.0	26.1	21.6	0.90	13.8	1.15	12	87
High P (a & b)	6.1	46.6	35.9	30.2	1.00	14.4	1.06	14	94

Table 3. Percentage of total inorganic phosphorus extracted by various solvents.

Soils	Reagents investigated.			
	2.5% HAc	Truog reagent	Lactate reagent	1% citric acid
	%	%	%	%
No P	0.5	1.2	0.05	1.5
Mod P (a & b)	2.0	1.5	0.03	4.9
High P (a & b)	3.5	2.8	0.1	5.1

B. Methods.

I. Addition of phosphate.

In this investigation, the amounts of phosphate added to the soils were considerably in excess of those normally used under field conditions. This was essential to enable the phosphate distribution among the several types of binding to be followed satisfactorily by chemical fractionation procedures.

It was calculated that an addition of 80 m.g. P/I00g soil would be adequate to ensure that detectable differences would occur in the major categories of bound phosphate in the soil.

For the addition of phosphate, a sample of soil weighing I60g, was spread evenly over a twelve - inch diameter clock - glass. The soil sample was moistened with I60 ml of an 800 p.p.m. solution of KH_2PO_4.

To avoid the development of localised concentrations of phosphate during the subsequent drying process, the soil sample was moistened as uniformly as possible with the phosphate solution. The drying was carried out at room temperature and during this time the soil was subjected to periodic mixing. When dry, the soil was transferred to a screw - topped jar and shaken end - over - end for several hours, to complete the mixing process.

The uniformity of distribution of phosphate throughout the soil was tested for by determining total phosphate on a number of samples withdrawn at random from the air - dried soil. The results shown in Table 4 indicate that the procedure used was successful in providing an acceptably even distribution of phosphate throughout each soil.

Table 4. Amounts of total phosphate estimated prior to, and immediately following, phosphate addition.

Soil	Before phosphate addition.	After phosphate addition. Triplicates.			Amount of phosphate added to air-dry soil - by difference.
	mg P%	mg P%	mg P%	mg P%	mg P%
No P	228	308	311	313	83
Mod P (a)	358	447	449	448	90
Mod P (b)	315	409	402	405	90
High P (a)	388	468	465	471	80
High P (b)	372	460	459	459	87

2. Experimental Design.

As it was desired to obtain a continuous record of the trends of phosphate reactions in the phosphate - treated soils, it was necessary to decide on the most suitable method to allow for simple periodic sampling. Rather than sample at intervals from the bulk soil as prepared above it was decided to partition the bulk sample into separate 20g. subsamples. This weight of soil was considered adequate to allow the full range of chemical determinations required at each two - monthly period.

(i) Condition of storage of phosphated - soils.

Each 20g subsample was stored throughout the experiment in a I30 ml screw - topped jar. Water in sufficient amount (8 - I0 ml) to provide adequate moisture to facilitate chemical reactions between the soils and the added phosphate was then added.

At intervals throughout the experiment the jars were weighed to check on possible moisture losses by evaporation.

To avoid the possibility that microbial activity might be stimulated to unnatural levels in the presence of the high phosphate addition made to the soils, it was considered desirable to limit microbial growth as far as possible. To this end a few drops of a toluene - ether mixture, as employed by Kurtz, De Turk and Bray (I946) was added to the soils in the jars, following the water addition. Also the jars were stored throughout the investigation in a cupboard away from light.

(ii) Additional treatments included in the experiment.

In addition to the soils prepared as described in I and 2(i) above, controls were included into the experiment by preparing two extra twenty gram samples of each soil. One sample was treated with phosphate as above but not subjected to the toluene - ether treatment. The other sample was removed from the bulk soil sample before phosphate treatment and was subjected to toluene - ether treatment.

These control samples of each soil were stored throughout the duration of the experiment and used as a source of comparative data at the conclusion of the investigation.

Summary of Experimental layout.

The various treatments accorded the soils in this investigation are summarised below.

(a) Five samples of each soil, treated with phosphate and subjected to the toluene - ether treatment.

(b) One sample of each soil, treated with phosphate but not subjected to the toluene - ether treatment.

(c) One sample of each soil, not treated with phosphate but treated with the toluene - ether mixture.

3 Microbial Count Technique.

The effectiveness of the toluene - ether additions on limitation of microbial populations was investigated by an agar plate method.

(i) Media.

Two agar media were used for the estimation of microbial numbers

(a) A soil extract agar.

This medium consisted of 4g agar, 0.Ig dipotassium phosphate, 0.04g sucrose and 800 ml of a soil extract.

(b) Czapek Dox agar.

This agar was made from I0g of Czapek agar and 200 ml of water.

Both media were sterilised under pressure before they were used.

(ii) Preparation of soil for microbial count.

A preliminary experiment indicated that I/I,000,000 dilution of the soil was a satisfactory dilution for plating out.

The soil was shaken with sterilised water for I6 hours after which a series of dilutions were prepared until the I/I,000,000 was reached.

(iii) Pouring of plates.

Five replicates for each medium and soil were plated at the IO^{-6} dilution.

Care was taken during the plate pouring to avoid aerial contamination.

From the IO^{-6} dilution, I ml of the freshly - agitated suspension was withdrawn with a sterile pipette and transferred to the appropriate petri dish.

Approximately I2 ml of the liquefied medium was poured into the appropriate petri dish. Immediately following pouring the petri dishes were rotated several times in a clockwise and then anti - clockwise direction to ensure adequate mixing of the agar and soil suspension.

When the agar had solidified, the petri dishes were inverted prior to incubation.

(iv) Incubation and plate counting.

The plates were incubated at 28°C for IO days. Colony counts of the plates were made after 5 and IO days incubation.

All plates showing more than 300 colonies and less than IO colonies were discarded. The plates were counted with the naked eye or with low - power magnification. The final total counts were corrected to an oven - dry soil basis before comparison.

.4 Analytical Procedures.

(a) Determination of phosphate.

Unless where otherwise stated, the method used for the estimation of phosphate in solution was that of Dickman and Bray (1940).

(b) Determination of total phosphate.

The perchloric acid digestion method of Sherman (1942) was used with the modified phosphate determination of Schricker and Dawson (1939), as reported by Bray and Kurtz (1945).

(c) Procedure for phosphate fractionation.

The general procedure followed as indicated in Fig 1.

Preliminary experiments were carried out to ascertain the most suitable soil: extractant ratios to be used both for the extraction of aluminium - bound phosphate and iron - bound plus aluminium - bound phosphate. The results of this work are shown in Tables 5 and 6.

It was concluded that a 1:800 ratio would provide a satisfactory measure of aluminium - bound phosphate without recourse to correction for resorption by free iron oxides. This is in accord with the findings of Fife (1962) for a Stratford sandy loam.

A ratio of 1:400 was adopted for the determination of iron - bound plus aluminium - bound phosphate on the basis of these preliminary experiments.

The extraction time adopted for aluminium - bound phosphate (16 hours) and for iron - bound plus aluminium - bound phosphate (24 hours) were also established by preliminary experiments.

For the determination of aluminium - bound phosphate the boric acid procedure of Kurtz (1942) was used to eliminate fluoride ion interference during colorimetric determination of the phosphate extracted.

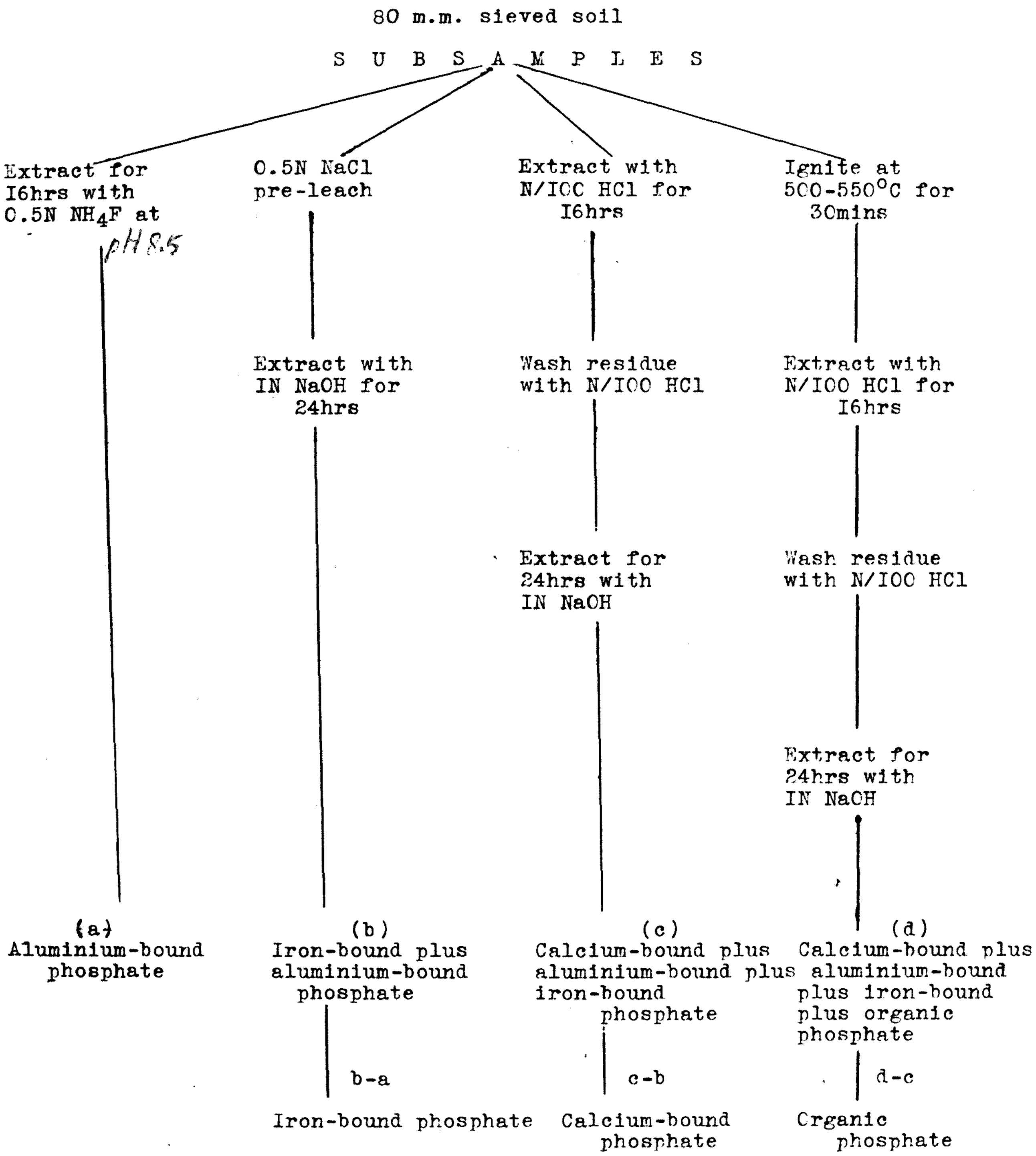
For the determination of organic phosphate a comparison was made between the method shown in Fig 1 and the method of Williams and Stewart (1941). In the latter method, organic phosphate is determined as the difference between 1N H_2SO_4 extraction of ignited and unignited soil samples. Table 7 shows that similar values for organic phosphate were obtained by both procedures. The method outlined in Fig 1 was adopted for all subsequent work.

(d) Determination of the reciprocal of absorption.

The method proposed by Russell (1963) was used.

A 0.2g sample of soil was shaken with 20 ml of a 5 p.p.m. P solution for four days. The phosphate remaining in solution after this period was determined and the reciprocal of absorption was calculated as the ratio of the amount of soil used, to the amount of phosphate (in m.g. P) absorbed by the soil.

Figure 1. Graphical representation of the phosphate fractionation procedure used.



80 m.m. sieved soil

SUBSAMPLES

Extract for I6hrs with $0.5N\ NH_4F$ at pH 8.5

(a)
Aluminium-bound phosphate

0.5N NaCl pre-leach

Extract with IN NaOH for 24hrs

(b)
Iron-bound plus aluminium-bound phosphate

b-a

Iron-bound phosphate

Extract with N/I00 HCl for I6hrs

Wash residue with N/I00 HCl

Extract for 24hrs with IN NaOH

(c)
Calcium-bound plus aluminium-bound plus iron-bound phosphate

c-b

Calcium-bound phosphate

Ignite at 500-550°C for 30mins

Extract with N/I00 HCl for I6hrs

Wash residue with N/I00 HCl

Extract for 24hrs with IN NaOH

(d)
Calcium-bound plus aluminium-bound plus iron-bound plus organic phosphate

d-c

Organic phosphate

Table 5. Amounts of Aluminium - bound phosphate extracted by 0.5M NH_4F at pH 8.5, for a range of soil/extractant ratios.

Soil	Soil/Extractant ratio.				
	1:100	1:200	1:400	1:800	1:1600
	mg P%	mg P%	mg P%	mg P%	mg P%
No P	54.8	61.4	64.5	67.6	64.2
Mod P (b)	96.1	103.2	108.5	108.6	110.1
High P (b)	138.6	145.8	151.8	154.5	153.6

Table 6. Amounts of iron - bound and aluminium - bound phosphate extracted by 1 N NaOH for a range of soil/extractant ratios.

Soil	Soil/Extractant ratio.			
	1:100	1:200	1:400	1:800
	mg P%	mg P%	mg P%	mg P%
No P	44.4	56.8	58.4	57.4
Mod P (b)	93.4	103.2	104.7	103.3
High P (a)	133.4	159.3	164.8	162.1

Table 7. A comparison between the amounts of organically - bound phosphate estimated by two methods.

Soil	Method	
	0.01N HCl + 1N NaOH extraction (Fig. 1)	1N H_2SO_4 extraction (Williams and Stewart (1940)).
	mg P%	mg P%
No P	109.0	109.5
Mod P (b)	150.1	142.4
High P (a)	158.9	153.9

(e) Determination of total isotopically - exchangeable phosphate.

I Introductory.

The technique used in this investigation involved the introduction of three major modifications to existing methods for the estimation of total isotopically exchangeable phosphate in soils. These modifications were:-

(i) an extended period for chemical equilibration.

(ii) a change in the mode of addition of the radioactive phosphate solution.

(iii) an extended period for isotopic equilibration.

Most previous workers have encountered difficulties in the measurement of ^{3I}P - phosphate in solution after equilibration of a soil with water. To overcome this problem a variety of methods have been introduced.

Wiklander (I950) treated soils for 3 days with labelled mono - calcium phosphate solutions and studied the desorption of ^{32}P - phosphate on the soil into an unlabelled phosphate solution.

Gunnarsson and Fredrikson (I95I) used large amounts of ^{32}P - labelled phosphate during measurement of total isotopically exchangeable phosphate, so that the ^{3I}P - phosphate concentration in solution remained high enough to be accurately measured.

Mattingly (I957) has criticised the addition of orthophosphate to soils during measurement of total isotopically exchangeable phosphate because he contended that the distribution of phosphate on soil surfaces would be affected by the additions.

Russell, Rickson and Adams (I954) used a mixed ^{3I}P - and ^{32}P - phosphate solution during estimation of the E value but mentioned that the effect of absorption of ^{3I}P by the soil, on the E value depended on the exchangeability of the sorbed phosphate. If the exchangeability remained high then there was little or no effect on the E value obtained.

Other workers have attempted to increase ^{3I}P - phosphate in solution by a variety of methods. Talibudeen (I957) used 0.00I M NH_4 citrate plus 0.02 M KCl to alter the ratio of ^{3I}P soil: ^{3I}P solution. Barbier (I954) shook the soil with 0.0I5 N $CaSO_4$ before determination of total isotopically exchangeable phosphate.

In the present investigations adequate concentrations of ^{3I}P - phosphate were generated in the equilibrium solutions in the absence of added phosphate. The E values reported thus pertain to fully equilibrated systems.

The time allowed for *chemical* equilibrium to establish appears to vary with the experimenter. Talibudeen (I957) and Barbier (I954) used a 48 hour shaking period. Wiklander (I950) shook the soil for three days with a phosphate solution before measurement. McAuliffe, Hall and Dean (I948) and Seatz (I954) used a 4 day *chemical* equilibration period.

However, from work by Shao (priv.comm.) and preliminary experiments in this investigation (Fig 2) it appeared that, for the yellow - brown loams, true equilibrium would not be reached in periods shorter than 7 days. As a precautionary measure the *chemical* equilibration time was extended to I4 days in subsequent work.

Shao (priv.comm.) concluded that the period necessary for *isotopic* equilibrium in most soils was about 2 weeks. This finding is in agreement with work by Scott - Russell (I954) who showed (for one soil) that the required period for *isotopic* equilibrium was 49 days.

In the present study, a two week period was adopted for the establishment of isotopic equilibrium.

2. Procedure.

A 0.5g soil sample was shaken for two weeks with 5I ml of distilled water in a IOO ml centrifuge tube. After this period the soil was centrifuged down and I.5 ml of the supernatent solution was transferred to a 5 ml test - tube and activated with one drop of a high specific activity ^{32}P - phosphate solution. (supplied by Radiochem Centre, Arnestrom, England.) After thorough mixing, 0.5 ml of the radioactive solution was returned to the bulk soil/water system in the centrifuge tube. A further 0.5 ml of the radioactive solution was pipetted into a second centrifuge tube containing 49.5 ml of a dilute phosphate solution. This tube provided a system of equivalent activity, and obviated the need to apply isotopic decay corrections to the ^{32}P - phosphate in the soil/water systems. The dilute phosphate solution in this blank centrifuge tube prevented ^{32}P - phosphate from adsorbing on to the glass. Both centrifuge tubes were stoppered and shaken end over end for a further two weeks. The tubes were then centrifuged and the solutions counted using a liquid geiger counter (20th Century Electronics, Type M 6 H), assembled as shown in Plate I. The ^{3I}P - phosphate concentration was then determined on a aliquot of the solution which had been equilibrated with the soil.

Figure 2. Graph of count rate versus time for the determination of chemical equilibration period.

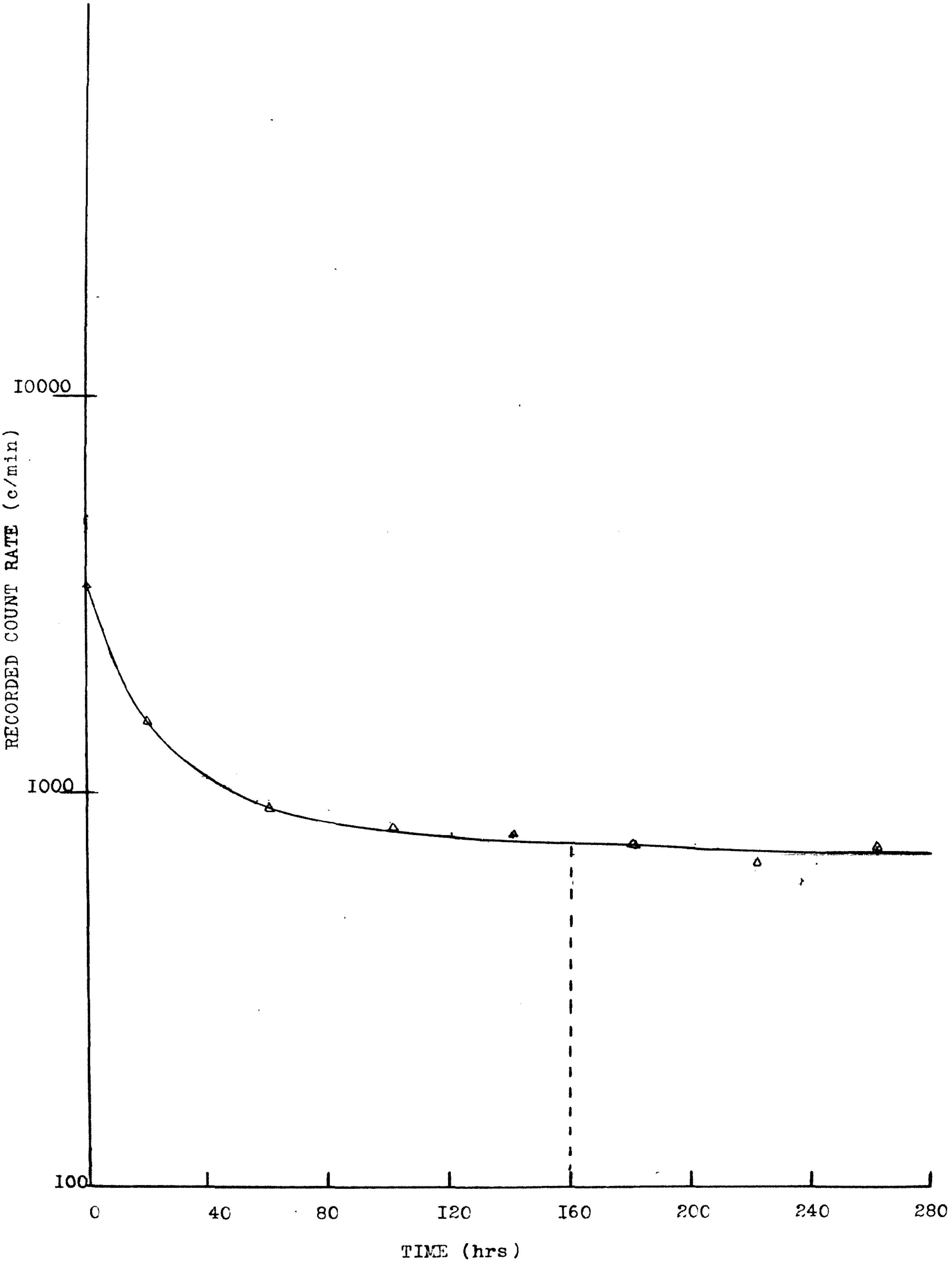
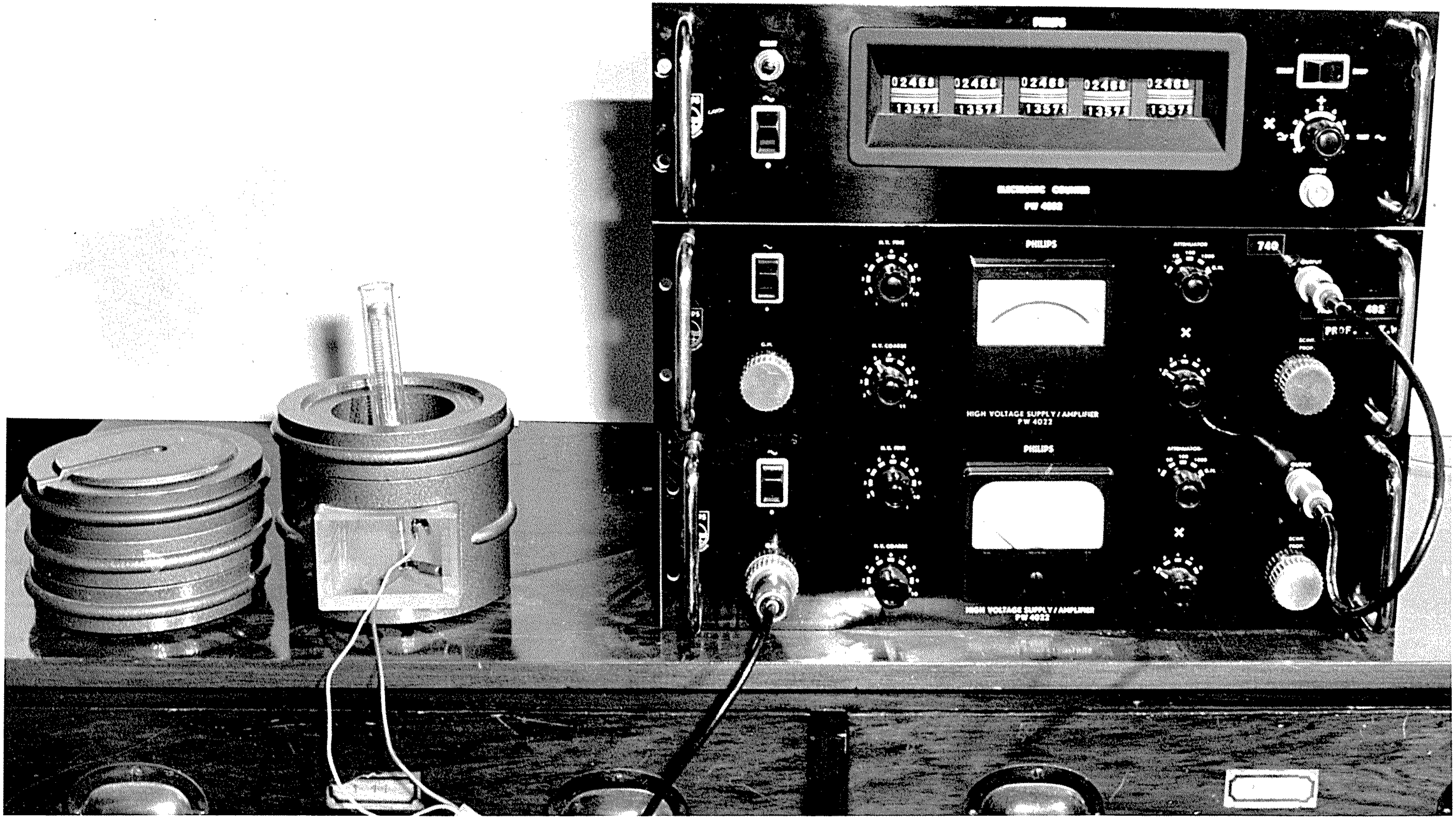

I0000
I000
I00
RECORDED COUNT RATE (c/min)
0
40
80
I20
I60
200
240
280
TIME (hrs)

Plate 1. Equipment assembled for radioactive solution counting during the determination of total isotopically exchangeable phosphate.


PHILIPS
PHILIPS
740
HIGH VOLTAGE SUPPLY / AMPLIFIER
PW 4022
PHILIPS
HIGH VOLTAGE SUPPLY / AMPLIFIER
PW 4022

3. <u>Calculation of total isotopically - exchangeable phosphate.</u>

The total isotopically - exchangeable phosphate in the soil was calculated as follows.

$$\frac{^{31}\text{P soil}}{^{32}\text{P soil}} = \frac{^{31}\text{P solution}}{^{32}\text{P solution}}$$

$$\text{therefore } ^{31}\text{P soil} = {}^{31}\text{P solution} \times \frac{^{32}\text{P soil}}{^{32}\text{P solution}}$$

$$= {}^{31}\text{P solution} \times \frac{\text{initial } ^{32}\text{P solution - final 320 solution}}{\text{final } ^{32}\text{P solution.}}$$

finally total isotopically exch P = ^{31}P soil ÷ ^{31}P solution.

In the above equations:-

^{31}P solution was the total amount of ^{31}P in solution in the equilibrated soil/solution system.

^{32}P initial was the count obtained for the blank tube.

^{32}P final was the count obtained in the equilibrium solution, after separation from the soil by centrifugation.

A Fortran programme, shown below, was devised for the I.B.M. I620 computer to enable a direct estimation of the total isotopically - exchangeable phosphate to be estimated directly from the experimental results.

PROGRAMME FOR
TOTAL ISOTOPICALLY EXCHANGEABLE PHOSPHATE

```
12 FORMAT (F5.0,F5.0,F6.4,F11.8,F5.0)
13 FORMAT (7H TOTP= ,F14.8,5H SOIL,I3)
50 I=1
80 READ 12,P32B,P32A,P31S,TAU,BKD
   IF(P32A-9999.) 60,32,32
60 P32A=P32A/(1.-P32A*TAU)
   P32A=P32A-BKD
   P32B=P32B/(1.-P32B*TAU)
   P32B=P32B-BKD
   TOTP=P31S*(P32B-P32A)/P32A
   TOTP=TOTP+P31S
   TYPE 13,TOTP,I
   I=I+1
   GO TO 80
32 IF (SENSE SWITCH 1 ) 50,33
33 STOP
   END
```

(f) Determination of simultaneous isotopic exchange kinetics.

I. Introductory

Until very recently, few attempts have been made to investigate the types of exchangeable phosphate in soils and the rates at which these types undergo exchange.

Seatz (I954) and Talibudeen (I957) noted, during measurement of total isotopically - exchangeable phosphate in soils, inflexion points in a graph of fractional activity in the soil against the log of time.

In a recent review Mattingly (I957) stated that in general, during isotopic exchange measurement, the activity in solution falls rapidly due to the exchange of surface ^{31}P - phosphate ions for added ^{32}P - phosphate ions, and then more slowly. This slow exchange of phosphate ions was attributed to recrystallisation processes in the soil or to rearrangement of lattice ions to give a structure with low free energy.

Weikel, Neuman and Feldman (I954) concluded that there were three different reactions involved during phosphate exchange on to synthetic hydroxyapatite. These reactions appeared to be:-

(a) a fast initial surface exchange of ions.

(b) diffusion of phosphate ions through the hydration layer at the surface.

(c) slow recrystallisation of the lattice.

Although reports are available on the exchange of phosphate on artificially prepared phosphates or hydroxides, (Rickson I95I, Olsen I952, Caro and Hill I956, Knight and Williams I956), and on to resins, (Wiklander I950, Russell, Rickson and Adams I954, Amer, Bouldin, Black and Duke I955), as previously mentioned little work has been done on the identification of types, and rates of reaction, of exchangeable phosphate in soils.

Furkert and Wilson (1962) reported a technique which enabled the exchange of water on to montmorillonite to be studied, and the curve resulting from a graphical plot of the log of the activity in solution against time, to be mathematically resolved into a number of types of exchanging water. Shao (1962) has applied this technique to the soil/phosphate systems and has demonstrated the presence of several characteristic types of exchangeable phosphate which go to make up the total isotopically - exchangeable phosphate in soils. Basically, Shao's technique embodies,

(a) the chemical equilibration stage in which the soil phosphate is brought to equilibrium with water using the apparatus depicted in Plate 2.

(b) the isotopic equilibrium stage in which, using the same apparatus, the drop in count rate of added high specific activity ^{32}P - phosphate is continuously recorded.

On attainment of isotopic equilibrium, the log of the recorded count rate is plotted against time and the resulting curve is subjected to the following mathematical treatment (Furkert (1962)).

If there are A grams of phosphate in solution and B grams of phosphate adsorbed onto the soil, and the rate at which phosphate is being adsorbed onto the soil is equal to R, then, as only the equilibrium state is being considered, the rate at which Phosphate ~~water~~ B is being desorbed from the soil is also equal to R. If the specific rate constant for the exchange is K i.e. $A \underset{K_b}{\overset{K_a}{\rightleftharpoons}} B$, then $R = K_b B$. At zero time, To counts are introduced into the circulatory system, and at time t there are x counts in the soil sample, then there will be (To - x) counts remaining in solution. The count rate is measured continuously and plotted against time. The resultant curve obtained from this plot has a slope equal to the rate of fall of the count rate in the geiger counter i.e. equal to $\frac{dT}{dt}$ where $T = (To - x)$ = count rate at time t. This curve gradually flattens out and eventually as the count rate falls to a steady value, the slope becomes equal to zero i.e. the count rate is no longer falling. Mathematical computation shows that, $\frac{T}{To} = \frac{A}{A + B} + \frac{B}{A + B} e^{\frac{-R(A + B)t}{AB}}$ .... (1)

When the count rate has fallen to a steady value, then this value will be the same as that for $t = \infty$, and if at $t = \infty$ $T = Z$ then from (1) since $e^{\frac{-R(A + B)t}{AB}} = o$

$$\frac{Z}{To} = \frac{A}{A + B} \quad .... (3)$$

If a graph is prepared of $\log_e T$ versus t, a curve levelling off to a steady value is obtained. This steady value may be subtracted from the rest of the curve, when the resultant graph will be a straight line. As this steady value is equal to $\frac{ToA}{A + B}$ the straight line will correspond to the exponential part of (1), and will be related to the exchangeable phosphate "B" on the soil. For if this line cuts the $t = 0$ axis at **x** then as $Z (= \frac{A\ To}{A + B})$ has been subtracted from the curve

$$x = \frac{To\ B}{A + B} \quad .... (7)$$

If there are two (or more) types of exchangeable phosphate then a total of two (or more) sloping lines will be obtained with intercepts x, y, w etc. As was derived earlier, the rate is equal to the specific rate constant multiplied by the amount of phosphate "B" on the soil.

i. e. $R = K_b B$ .... (2)

From (1) after the subtraction of the steady state value Z the new value T_1 for the count rate is given by

$$\frac{T_1}{To} = \frac{B}{A + B} e^{\frac{-R(A + B)t}{A\ B}}$$

which on substitution of the value for R from equation (2) gives

$$\frac{T_1}{T_0} = \frac{B}{A + B} \quad e^{\frac{-K_b (A + B)t}{A}}$$

which can be written as

$$\log_e \left(\frac{T_1}{T_0} \cdot \frac{(A + B)}{B}\right) = \frac{K_b (A + B)t}{A} \quad \ldots\ldots\ldots (6)$$

now $t_{\frac{1}{2}}$ is the time that the count rate takes to decrease to one - half of its original value, and thus equation (6) reduces to

$$\log_e \left(\tfrac{1}{2}\right) = \frac{-K_b(A + B)t_{\frac{1}{2}}}{A}$$

and as $- \log_e \frac{1}{2} = \log_e 2 \doteqdot 0.693$

therefore $K_b = \dfrac{A \times 0.693}{(A + B)t_{\frac{1}{2}}} \quad \ldots\ldots\ldots\ldots (4)$

If there are 2 (or more) types of exchangeable phosphate ("B", "C", "D" etc) in the soil, then equation (4) will apply to "B" where "B" is the type of phosphate that exchanges slowest with the phosphate in solution. To obtain a similar equation for "C" the faster - exchanging type of phosphate on the soil, it is considered that the rate of exchange of "C" is so much faster than that of "B" that the exchange of "C" with "B" and also with "A" is not important. Thus the equation for the determination of the specific rate constant K_c for the exchange of "C" with the rest of the water present will be obtained by replacing B by C and A by (A + B) in equation (4) leading to:

$$K_c = \frac{(A + B) \times 0.693}{(A+B+C)t_{\frac{1}{2}}} \quad \ldots\ldots\ldots\ldots (5)$$

where $t_{\frac{1}{2}}$ now refers to the half - time of exchange of the faster exchanging type of phosphate "C". If there are more types of water ("D", "E" etc) on the soil, their specific rate constants (K_d, K_e etc) will be given by the corresponding equations to equation (5) where C is replaced by D and (A + B) by (A + B + C) and so on. If the second line corresponding to the phosphate "C" cuts the t = 0 axis at y, then y will be related to the amount of activity and hence to the total amount of exchangeable phosphate "C". However all the activity apart from that in "C" is "A" and "B" (assuming that the rate of exchange of "B" with "A" is much less than that of "C" with "A") and only a fraction of this activity in "A" will be counted. Therefore to obtain the amount of water in C it will be necessary to multiply y by the ratio $\frac{A + B}{A}$. Then if B is replaced by C and A by (A + B) as in the derivation of equation (5) the equation

$$\frac{y}{To} \times \frac{(A + B)}{A} = \frac{C}{A + B + C} \quad \ldots\ldots\ldots (8)$$

is obtained. Similar reasoning leads to the equation

$$\frac{w}{To} \times \frac{(A + B + C)}{A} = \frac{D}{A + B + C + D} \quad \ldots\ldots\ldots (9)$$

where w is the intercept of the line corresponding to a third, even faster exchanging type of exchangeable phosphate with the t = 0 axis . The total amount of exchangeable phosphate in the soil is equal to the sum of "B", "C", "D" etc.

2. Method.

(i) Apparatus.

The apparatus consisted of a three - chambered glass circulatory system (Plate 2) fitted with a glass centrifugal pump. The pump was powered by an electric stirrer. As shown in Plate 3, this apparatus was connected to a power supply, ratemeter and strip - chart recorder.

(ii) Procedure.

At the commencement of the determination, a glass wool plug was loosely packed into the base of Chamber C (Plate 2) and about 4g of coarse, acid-washed sand was poured on top of this plug. Water was added to partly fill the circulatory system and a few drops of toluene were used to limit microbial growth during the experiment. A weighed amount of soil (usually 2g) was then added to Chamber C and the centrifugal pump was started.

The soil in Chamber C gradually settled to form a layer of even depth above the sand. The glass wool plug and the sand served to contain the soil in Chamber C and at the same time offered minimum impedance to the flow of water around the system. Finally, a liquid Geiger counter tube (DM 6) was placed in position in Chamber B and water was added to Chamber A to bring the final water level to that indicated in Plate 2.

Plate 2. Circulatory apparatus used for the equilibration of soil phosphate with water.

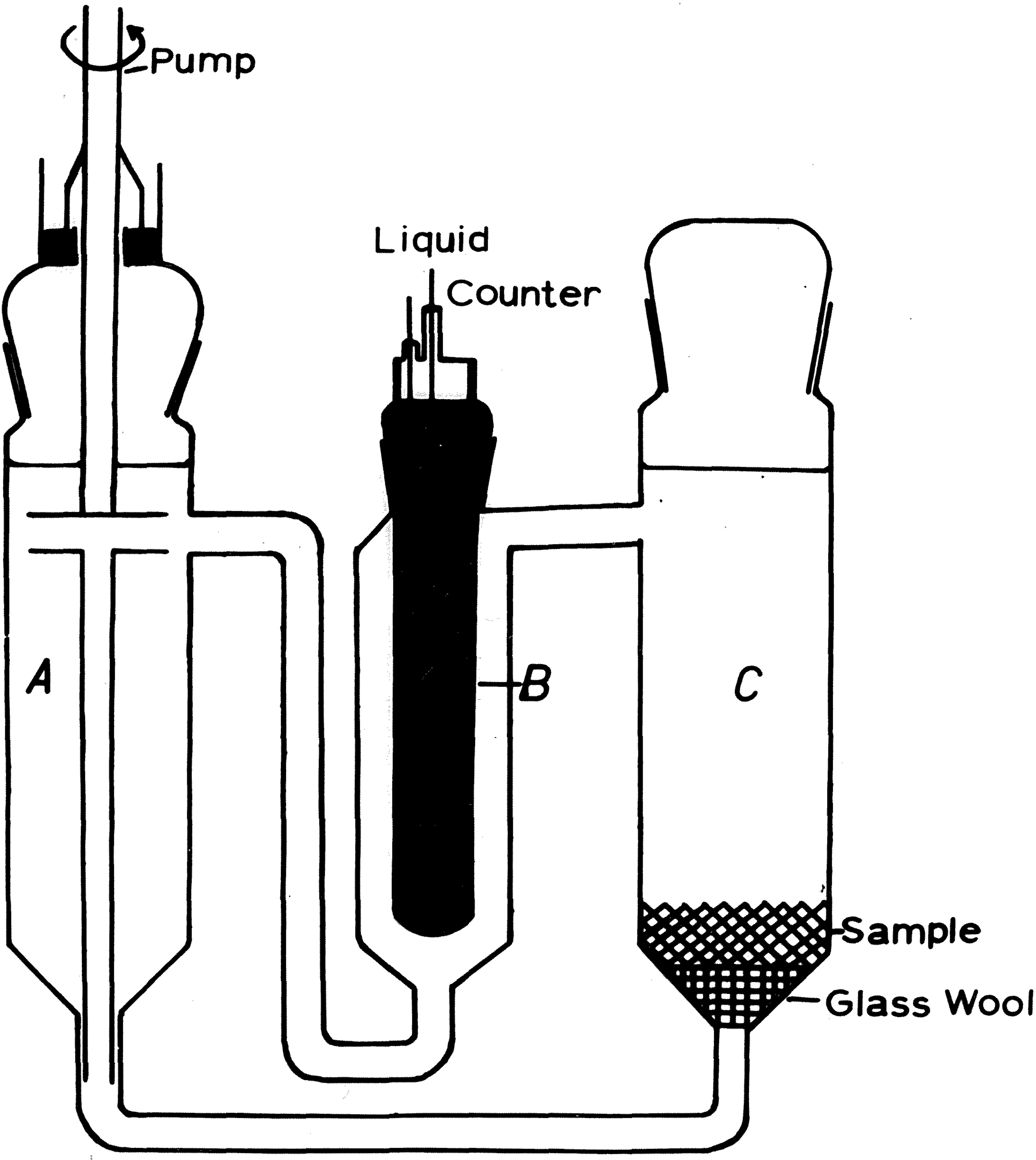
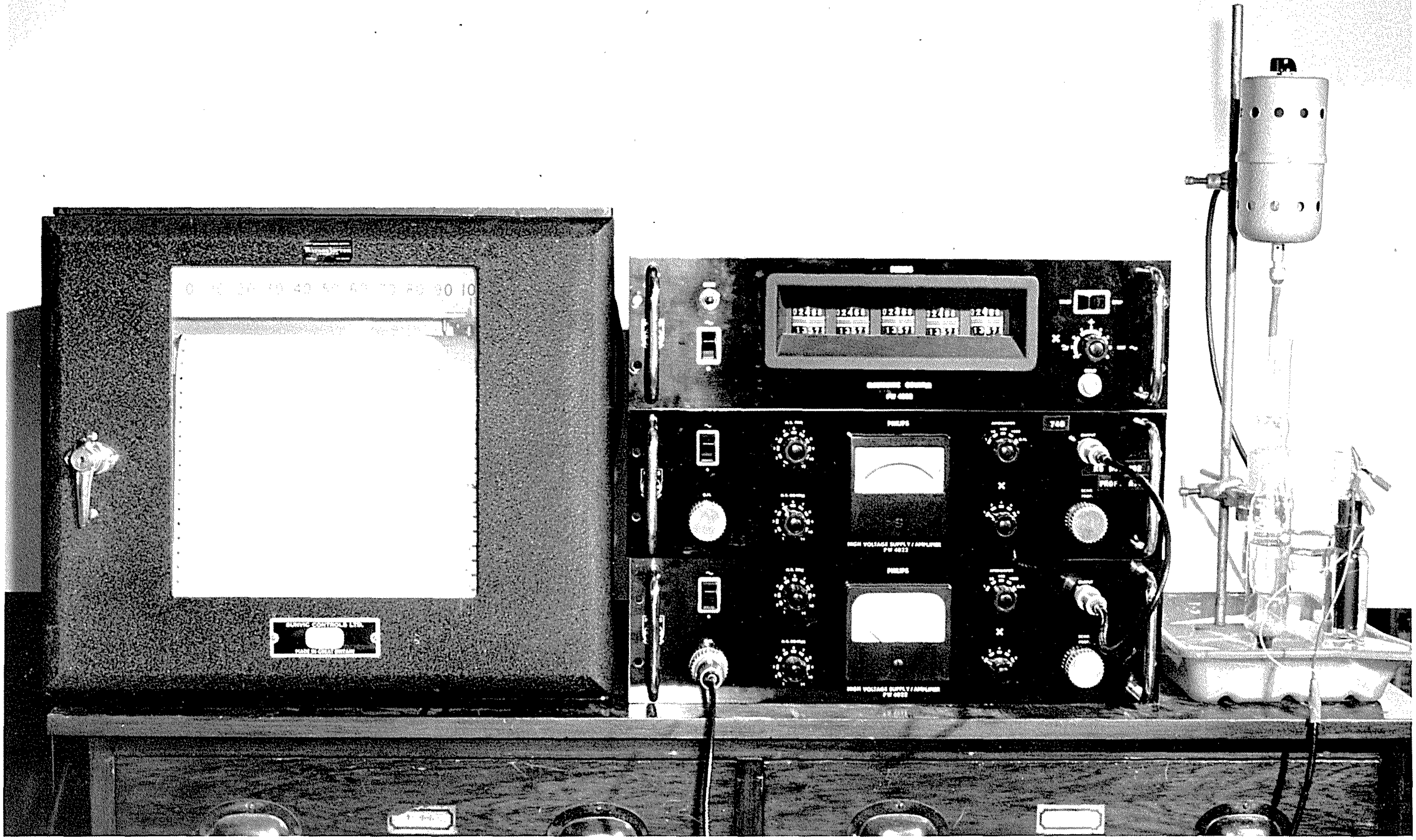

Pump
Liquid
Counter
A
B
C
Sample
Glass Wool

Plate 3. Equipment assembled for the simultaneous isotopic exchange kinetics investigation of soil phosphate.


0 10 20 30 40 50 60 70 80 90 10
SUNVIC CONTROLS LTD.
MADE IN GREAT BRITAIN
PHILIPS
HIGH VOLTAGE SUPPLY / AMPLIFIER
PW 4022
PHILIPS
HIGH VOLTAGE SUPPLY / AMPLIFIER
PW 4022
746

(iii) Determination of period required for chemical equilibration.

As it was essential to establish true chemical equilibrium in the systems prior to the exchange measurements a preliminary trial was carried out as follows.

The apparatus (Plate 2) was assembled, connected to the electronic equipment (Plate 3) and charged with water. The soil was not added at this stage. A drop of high specific activity ^{32}P - phosphate was added to chamber C and continuous counts of the activity in solution were made on the recorder. When these counts reached a steady maximum the soil sample was introduced into chamber C. The addition of soil caused a rapid decrease in the count rate, followed by a much slower decrease as the system tended towards equilibrium. Equilibrium was finally reached when the rate of fall of the recorded count was calculated as being fully accounted for by the decay rate of the isotope. As seen from Fig 2 the period for chemical equilibrium to be attained was approximately 7 days (I60 hrs.). It was realised that the rate of attainment of this equilibrium situation would depend to an extent on the circulation time of the apparatus. Because this was likely to vary between experiments the chemical equilibration time adopted was I4 days.

(iv) Application of simultaneous isotopic exchange kinetics (SIEK) Technique.

The apparatus was assembled as in Plate 2 with the glass wool plug, sand and soil in chamber C and the geiger counter in chamber B, the centrifugal pump was started and the system was left for I4 days to enable chemical equilibration to be reached. After this period the apparatus was connected to the electronic counting equipment as shown in Plate 3. A background count was obtained and then a drop of high specific activity ^{32}P - phosphate solution was added into chamber C. Continuous counts of the activity of the circulating solution were made on the recorder until calculation revealed that the fall in count rate with time was due only to the decay of the isotope and not to further exchange. When this stage was reached the apparatus was disconnected from the electronic equipment and the ^{31}P - phosphate concentration was determined on an aliquot of the circulated equilibrium solution.

(v) Calculation.

Two alternative methods, both based on the same mathematical principles, were available for the calculation of results from the experimental data. These alternatives were:-

(i) computation of the results by hand, using a graph of the logarithm of the count versus time.

(ii) use of a series of three computer programmes devised to increase the speed of calculation.

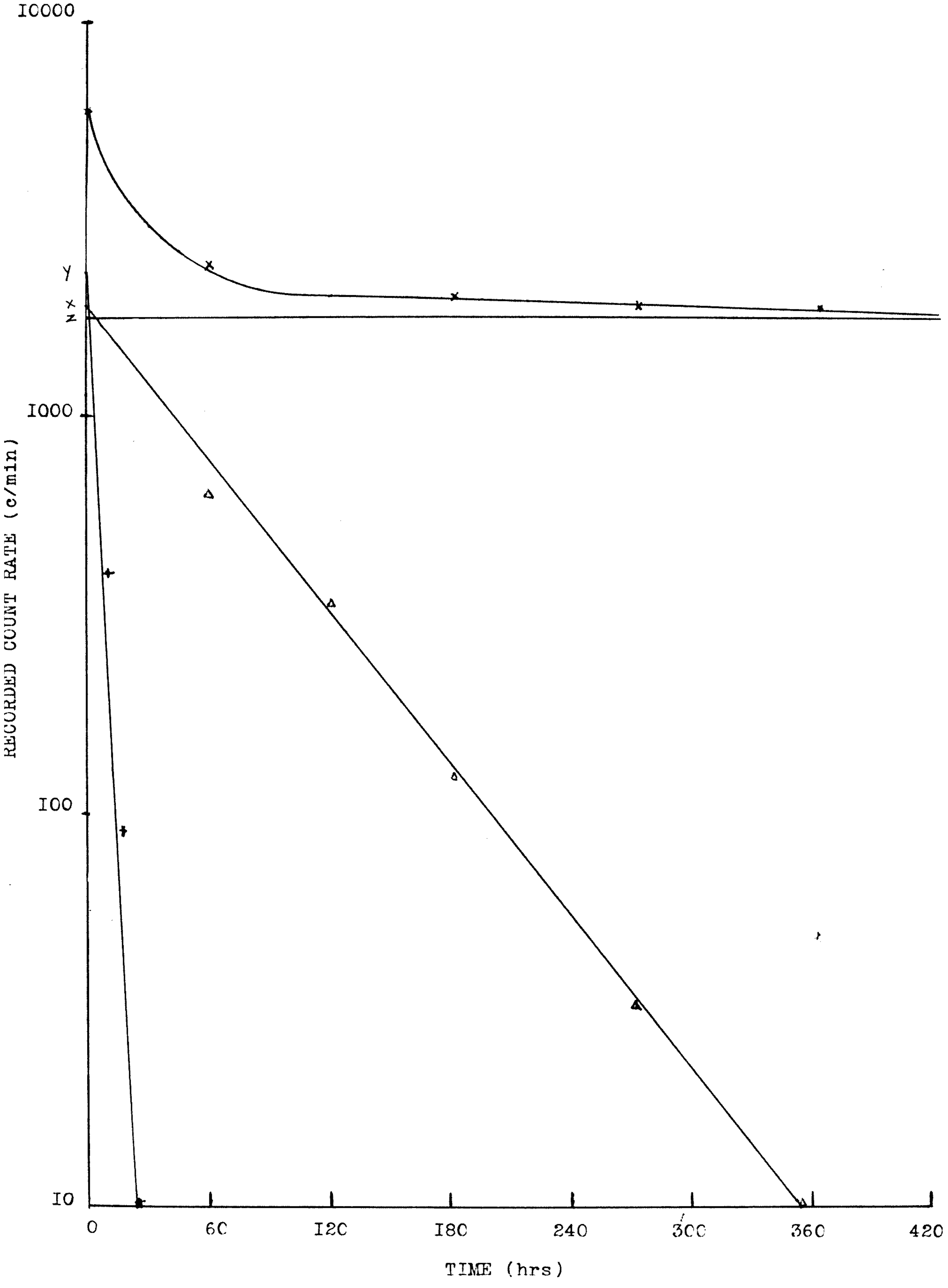
Apart from a worked example by method (i), shown below, the results were processed by (ii), which is considered the more reliable of the two methods.

(i) Example of computation of results by hand.

The results used in this computation were obtained from an experiment on the No - P soil sample which had been treated with phosphate (as described in Materials and Methods) and stored for 4 months prior to measurement.

A graph of count versus time was prepared using semi - logarithmic graph paper (Fig 3.).

Figure 3. Graph of count rate versus time for investigation of simultaneous isotopic exchange kinetics


I0000
Y
X
Z
I000
RECORDED COUNT RATE (c/min)
I00
I0
0
60
I20
I80
240
300
360
420
TIME (hrs)

From the experimental results,

^{3I}P solution = A = 0.0885mg P/g

From the graph (Fig 3),

X = intercept at count axis of Ist type of exchangeable phosphate.

= I862 c/min.

T_o = initial ^{32}P count

= 6330 c/min.

Now,

$$X = \frac{T_o\ B}{A + B} \quad \ldots\ldots (7)$$

therefore

$$B = \frac{X(A + B)}{T_o}$$

$$= \frac{AX + BX}{T_o}$$

$$= \frac{(0.0885 \times I862) + (I862)}{6330}$$

$$= \underline{0.0368 \text{ mg P/g}}$$

$t_{\frac{1}{2}}$ is obtained from the graph in the following manner. The intercept of X at the count axis is at I862 c/min. $t_{\frac{1}{2}}$ is the time taken for one half of "B" to exchange i.e. The time taken for the count rate to drop to 93I c/min. From the graph 93I c/min corresponds to 55 hours for this type of exchangeable phosphate, and this time value is regarded as the half life of "B"

i.e. $\underline{t_{\frac{1}{2}} = 55 \text{ hrs}}$.

Now, the rate constant (K_b) for B is calculated as,

$$K_b = \frac{A \times 0.693}{(A+B)\ t_{\frac{1}{2}}} \quad \ldots\ldots (4)$$

$$= \frac{0.0885 \times .0693}{(0.0885 + 0.0368)55}$$

$$= 0.00888$$

$$= \underline{0.888 \times IO^{-2}}$$

Also for the second type of exchangeable phosphate (C) the intercept on the count axis is at Y

$$\frac{Y}{T_o} \times \frac{(A + B)}{A} = \frac{C}{A+B+C} \quad \ldots\ldots \quad (8)$$

$$\text{i.e} \quad \frac{2700}{6330} \times \frac{(0.0885 + 0.0368)}{.0885} = \frac{C}{0.0885 + 0.0368 + C}$$

solving for C

therefore $\underline{C = 0.I9I04}$

The half life for C is obtained from the graph in a similar manner to that for B.

$$\underline{t_{\frac{1}{2}} = 2.6\text{hrs.}}$$

Also, the rate constant (K_c) for C is calculated as,

$$K_c = \frac{(A+B) \times 0.693}{(A+B+C)t_{\frac{1}{2}}} \quad \ldots\ldots \quad (5)$$

$$= \frac{(0.0885 + 0.0368) \times 0.693}{(0.0885 + 0.0368 + 0.I9I04) \times 2.6}$$

$$= \underline{0.I0558}$$

(ii) <u>Calculation by computer method</u>.

The three computer programmes used for the calculation of results from the experimental data are shown below.

PROGRAMME ONE

```
   DIMENSION COUNT(160),TIME(160),DIFF(150)
98 FORMAT (F10.5,F7.2,I4,F10.5)
13 FORMAT (2I3)
99 FORMAT (F6.0,F6.0)
12 FORMAT (F10.5,2X,F7.2,2X,I4)
15 FORMAT (4H Z= ,E14.8)
18 FORMAT (6X,F10.6,F11.8,F6.0)
   READ 18,AMBDA,TAU,BKD
   I=1
33 READ 99,COUNT(I),TIME(I)
   IF(TIME(I)-99999.) 30,31,32
32 STOP
30 COUNT(I)=COUNT(I)/(1.-COUNT(I)*TAU)
   G=AMBDA*TIME(I)
   R=2.7183**G
   COUNT(I)=COUNT(I)*R
   COUNT(I)=COUNT(I)-BKD
   COUNT(I)=LOG(COUNT(I))/2.3026
   TYPE 12,COUNT(I),TIME(I),I
   I=I+1
   GO TO 33
31 ACCEPT 13,K,L
   SUM=0.
   DO 14 I=K,L
14 SUM=SUM+COUNT(I)
   G=L-K+1
   AMEAN=SUM/G
   TYPE 15,AMEAN
   AMEAN=AMEAN*2.3026
   AMEAN=EXP(AMEAN)
   DO 16 I=1,K
   COUNT(I)=COUNT(I)*2.3026
   COUNT(I)=EXP(COUNT(I))
16 COUNT(I)=COUNT(I)-AMEAN
   DO 94 I=1,K
94 COUNT(I)=LOG(COUNT(I))/2.3026
   I=2
93 IF(K-I) 32,92,97
97 DIFF(I)=(COUNT(I-1)-COUNT(I))/(TIME(I)-TIME(I-1))
   PUNCH 98,COUNT(I),TIME(I),I,DIFF(I)
   I=I+1
   GO TO 93
92 STOP
   END
```

PROGRAMME TWO

```
   DIMENSION COUNT(130),TIME(130),DIFF(120)
29 FORMAT (I4)
98 FORMAT (F10.5,F7.2,4X,F10.5)
75 FORMAT (F10.5,2X,I4)
 4 FORMAT (2I3)
36 FORMAT (2E14.8)
11 FORMAT (4H X= ,E14.8,13H HALF LIFE= ,F10.5)
15 FORMAT (4H Y= ,E14.8,13H HALF LIFE= ,F10.5)
18 FORMAT (4H W= ,E14.8,13H HALF LIFE= ,F10.5)
21 FORMAT (4H Q= ,E14.8,13H HALF LIFE= ,F10.5)
   READ 29,J
   I=2
   K=1
81 IF (K-J) 8,82,32
 8 READ 98,COUNT(I),TIME(I),DIFF(I)
   TYPE 75,DIFF(I),I
   I=I+1
   K=K+1
   GO TO 81
82 M=0.
   ACCEPT 4,K,L
28 FCTR1=L-K+1
   SUMXY=0.
   DO 5 I=K,L
 5 SUMXY=SUMXY+COUNT(I)*TIME(I)
   SUMX=0.
   DO 6 I=K,L
 6 SUMX=SUMX+TIME(I)
   SUMY=0.
   DO 7 I=K,L
 7 SUMY=SUMY+COUNT(I)
   SUMX2=0.
   DO 88 I=K,L
88 SUMX2=SUMX2+TIME(I)*TIME(I)
   ANUM=FCTR1*SUMXY-SUMX*SUMY
   ADEN=FCTR1*SUMX2-SUMX*SUMX
   AX=ANUM/ADEN
   ANUM=SUMX2*SUMY-SUMX*SUMXY
   B=ANUM/ADEN
   M=M+1
   IF (M-1) 32,9,10
 9 X=B
   BTHF=.301/AX
   TYPE 11,X,BTHF
   PUNCH 36,X,BTHF
   GO TO 12
10 IF (M-2) 32,13,14
13 Y=B
   CTHF=.301/AX
   TYPE 15,Y,CTHF
   PUNCH 36,Y,CTHF
   GO TO 12
14 IF (M-3) 32,16,17
```

PROGRAMME TWO (CONTD)

```
16 W=B
   DTHF=.301/AX
   TYPE 18,W,DTHF
   PUNCH 36,W,DTHF
   GO TO 12
17 IF (M-4) 32,19,19
19 Q=B
   ETHF=.301/AX
   TYPE 21,Q,ETHF
   PUNCH 36,Q,ETHF
   GO TO 12
12 IF(K-3) 32,32,25
25 DO 27 I=2,K
   S=AX*TIME(I)+B
   S=S*2.3026
   S=EXP(S)
   COUNT(I)=COUNT(I)*2.3026
   COUNT(I)=EXP(COUNT(I))
27 COUNT(I)=COUNT(I)-S
   DO 96 I=1,K
   IF(COUNT(I)) 49,49,96
49 COUNT(I)=1.001
96 COUNT(I)=LOG(COUNT(I))/2.3026
   I=K
74 IF(I-1) 32,72,73
73 DIFF(I)=(COUNT(I-1)-COUNT(I))/(TIME(I)-TIME(I-1))
   TYPE 75,DIFF(I),I
   I=I-1
   GO TO 74
72 ACCEPT 4,K,L
   GO TO 28
32 STOP
   END
```

PROGRAMME THREE

```
   DIMENSION COUNT(10)
64 FORMAT (21H CHECK SENSE SWITCHES)
37 FORMAT (4H C= ,F10.8,2X,5H KC= ,E14.8)
34 FORMAT (4H B= ,F10.8,2X,5H KB= ,E14.8)
41 FORMAT (4H E= ,F10.8,2X,5H KE= ,E14.8)
39 FORMAT (4H D= ,F10.8,2X,5H KD= ,E14.8)
87 FORMAT (2E14.8)
21 FORMAT (F7.5,F8.5,I2)
36 FORMAT (4F6.4,4F8.5)
   READ 21,P,A,M
   COUNT(1)=P
   COUNT(1)=COUNT(1)*2.3026
   COUNT(1)=EXP(COUNT(1))
   TYPE 64
   PAUSE
   IF (SENSE SWITCH 1 ) 51,52
51 READ 87,X,BTHF
   X=2.3026*X
   X=EXP(X)
   IF (SENSE SWITCH 2 ) 53,52
53 READ 87,Y,CTHF
   Y=2.3026*Y
   Y=EXP(Y)
   IF (SENSE SWITCH 3 ) 54,52
54 READ 87,W,DTHF
   W=2.3026*W
   W=EXP(W)
   IF (SENSE SWITCH 4 ) 55,52
55 READ 87,Q,ETHF
   Q=2.3026*Q
   Q=EXP(Q)
52 B=X*A/(COUNT(1)-X)
   CB=A*0.693/((A+B)*BTHF)
   TYPE 34,B,CB
   IF(M-1) 32,35,46
32 STOP
46 FN=(A+B)/A
   C=Y*FN*(A+B)/(COUNT(1)-(Y*FN))
   CC=(A+B)*0.693/((A+B+C)*CTHF)
   TYPE 37,C,CC
   IF(M-2) 32,35,38
38 FN=(A+B+C)/A
   D=W*FN*(A+B+C)/(COUNT(1)-(W*FN))
   CD=(A+B+C)*0.693/((A+B+C+D)*DTHF)
   TYPE 39,D,CD
   IF (M-3) 32,35,40
40 FN=(A+B+C+D)/A
   E=Q*FN*(A+B+C+D)/(COUNT(1)-(Q*FN))
   CE=(A+B+C+D)*0.693/((A+B+C+D+E)*ETHF)
   TYPE 41,E,CE
35 STOP
   END
```

Summary of computer programmes.

Programme I.

The data input to this programme consisted of; the recorded counts (c/min) and the corresponding times (hrs), together with the corrections for coincidence (TAU, in hrs), decay (AMBDA, in hrs) and background (BKD, in c/min). The corrected counts were converted to logarithms and typed out on the console typewriter. A survey of this information revealed the point at which the count no longer fell with time. The index of this point (K) and the index for the last count (L) were then fed into the computer via the console typewriter. An average (Z) for the points from K to L was obtained and the remaining points (1⟶K) were punched, ready for the input for Programme 2.

Programme 2.

The data input for this programme consisted of the punched data from Programme I together with a value, J, which informed the computer of the total number of counts to be read. The differences between the log counts were typed out and a decision was made as to where these differences showed a sudden increase. The index point at which the change was noted (K) and the index number of the last count (L) were typed into the computer. The points from K to L were subjected to a least squares best fit treatment and the extrapolated intercept of this line, at the count axis was derived (X). The half life for this Ist type (B) of exchangeable phosphate was calculated and the programme repeated the above procedure for the successive types of exchangeable phosphate discernible, (viz C, D, E etc). The count axis intercepts (X, Y, W etc) for the types of exchangeable phosphate resolved, and their respective half - lives, were punched out ready for Programme 3.

Programme 3.

The input data to this programme consisted of the data from Programme 2 together with the log of the first corrected count from Programme I (P), ^{3I}P - phosphate in solution at equilibrium (A, in mg P/g) and the number of straight lines resolved in Programme 2 (M). The amounts of the types of exchangeable phosphate (B,C, D etc) and their corresponding rates of reaction were computed and typed out via the console typewriter.

———oOo———

III. Results and Discussion.

A. Microbial Counts.

From Table 8 it is evident that the Czapek agar always supported a higher microbial population than the soil extract agar, thereby indicating the favourable nature of the Czapek medium for microbial growth. The results suggest that the toluene - ether treatment, adopted in the main experiment for control of microbial action, has induced larger microbial populations than existed in the untreated soils.

Since, however, the agar - plate procedure provides something approaching optimum conditions for microbial growth and spore germination it is unlikely that the relative populations found by this procedure are a true indication of those existing in the soils over the IO month duration of the main experiment. Further, it is unlikely that, during the main experiment, there would have been any major changes in the distribution of the forms of phosphate as a result of microbial action. The evidence from Table 8, contrary to anticipated, implies that the toluene - ether treatment has created an environment favourable rather than inimical to microbial growth.

A point of further interest, evidenced in Plate 4, is that actinomycetes were the predominant microbial group surviving the toluene - ether treatment, whereas bacterial colonies dominated in the cultures prepared from untreated soils.

Table 8 Microbial Counts.

Soil	Treatment	Growth medium.	
		CZAPEK Agar	Soil extract agar
		Count	Count
High P (a)	Toluene - ether	71×10^6	34×10^6
"	Nil	43 " "	30 " "
High P (b)	Toluene - ether	46 " "	25 " "
"	Nil	24 " "	15 " "

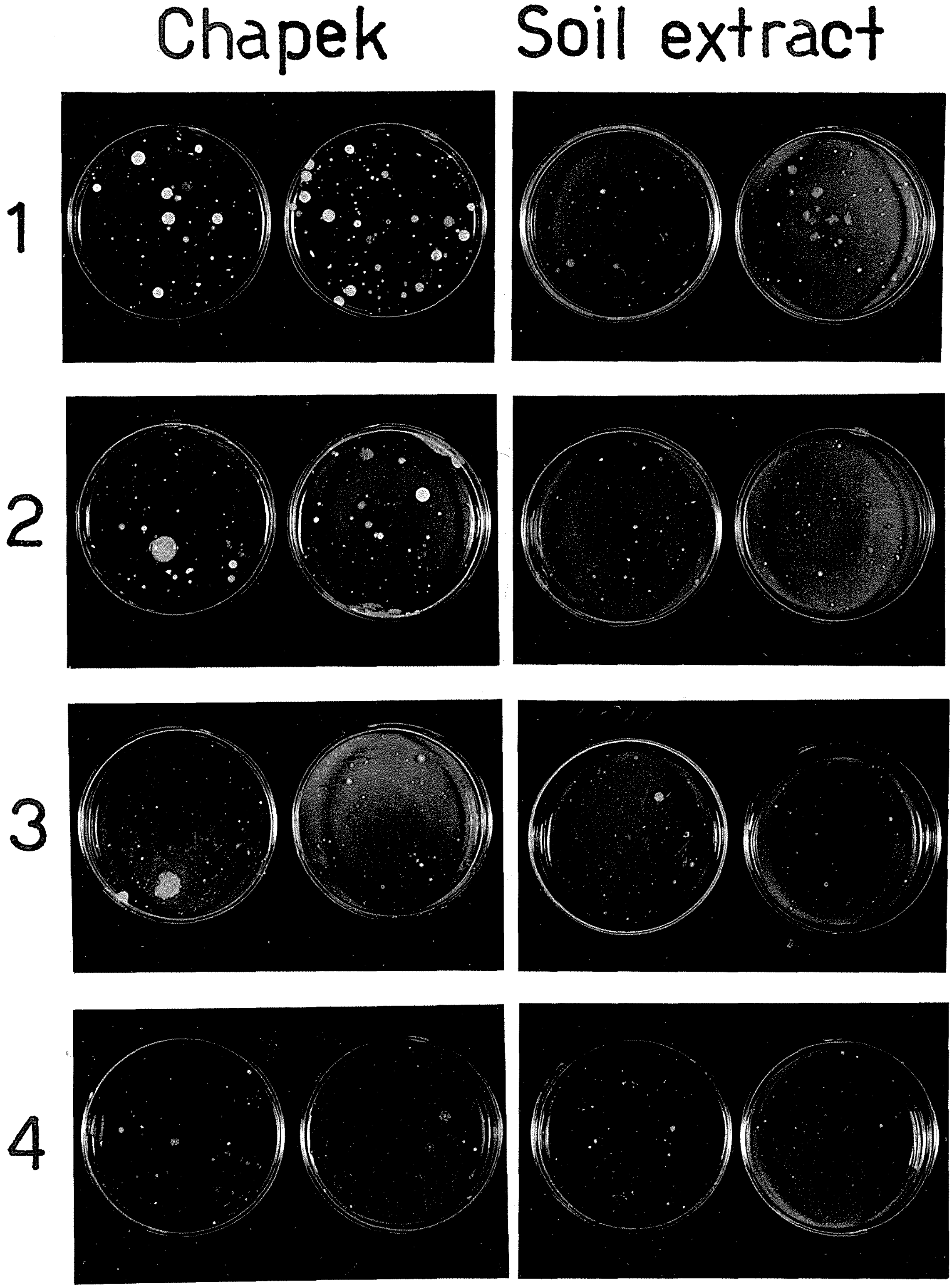
Plate 4. Colony counts from toluene - ether treated and untreated soils.

1 - Soil High P (a), Toluene - ether treated.

2 - Soil High P (a), untreated.

3 - Soil High P (b), toluene - ether treated.

4 - Soil High P (b), untreated.


Chapek
Soil extract
1
2
3
4

B. Phosphate fractionation.

(i) Total phosphate.

The results for the total phosphate contents of the soils before and after phosphate addition are presented in Table 9. These results show that all the soils are high in total phosphate before the phosphate addition at the commencement of the experiment. Also it is shown that the phosphate addition made at the commencement of the experiment increased total phosphate values by amounts within the range 83 - 90 mg P per cent. The discrepancy between the amount added and that recovered was not unexpected as the procedure, previously described, for the phosphate addition did not preclude the possibility of the added phosphate adhering to the clock glass on which the mixing of soil and phosphate solution was carried out.

Table 9 Total phosphate in soils prior to, and following phosphate addition.

Soil	Total P in original soil.	Total P in phosphated soils.	added P determined by difference	P actually added (converted to oven dry soil basis	Difference between P added and P recovered by analysis
	mg P%	mg P%	mg P%	mg P%	mg P%
No P	228	311	83	89	- 6
Mod P (a)	358	448	90	89	+ 1
Mod P (b)	315	405	90	89	+ 1
High P (a)	388	468	80	88	- 8
High P (b)	372	459	87	90	- 3

(ii) Calcium -, ÷ Iron -, ÷ Aluminium -, ÷ organically - bound phosphate.

The results for the amounts of phosphate present in the sum of Ca -, ÷ Fe -, ÷ Al -, ÷ organically - bound phosphate fractions, the increase in these fractions following the addition of phosphate, and a comparison of these values with total phosphate content, are shown in Tables IO, II, and I2.

It is evident from Tables IO and II that the sum of the Ca -, ÷ Fe -, ÷ Al -, ÷ organically bound phosphate fractions, accounts for all the added phosphate and remained sensibly constant throughout the experimental period. This demonstrates that during the storage period there was no conversion of added phosphate to forms not extractable by the reagents used; this applies to the "non - sterilised" sample (control 2) as well as to the toluene - ether treated samples. It is also evident from the values for "control I", that a IO month period of moistening of any one soil in the presence of toluene - ether, resulted in no change in the sum of these phosphate fractions.

Table I2 shows that the sum of Ca -, ÷ Al -, ÷ Fe -, ÷ organically - bound phosphate is lower than the total phosphate values in all the soils prior to the phosphate addition, indicating the presence of a difficultly - soluble inorganic phosphate fraction. This fraction, which is reasonably constant in amount and may be designated as "occluded" phosphate, evidently has not been supplemented during the period of storage of the soils even in the presence of added phosphate.

Table 10 Amounts of Ca -, + Fe^- + Al - + Organically - bound phosphate in the soils prior to, and at intervals following, phosphate addition.

Soil	Before Addition of phosphate	Time interval from commencement of experiment			
		2 months		10 months	
		Soil Treatment			
		phosphate added in presence of toluene - ether	Phosphate added in presence of toluene-ether	phosphate added in absence of toluene-ether (Control 2)	No phosphate added Toluene - ether treated only (Control 1)
	mg P%	mg P%	mg P%	mg P%	mg P%
No P	181	269	285	285	193
Mod P (a)	309	403	408	387	309
(b)	281	363	339	387	296
High P (a)	343	427	425	422	326
(b)	334	417	404	408	335

Table 11. Recovery of total added P in the sum of the Ca -, + Fe-, + Al- + organically - bound phosphate fractions.

Soil	Actual amount Phosphate added.	Amount Phosphate recovered after 2 mths storage.	Discrepancy between amount added and amount recovered in these fractions
	mg P%	mg P%	mg P%
No P	89	88	- 1
Mod P (a)	89	94	+ 5
(b)	89	81	- 8
High P (a)	88	84	- 4
(b)	90	83	- 7

Table 12. Comparison between total phosphate content of soils and amount of Ca-+ Fe-, + Al - + organically - bound phosphate before P addition.

Soil	Total P value	Ca-, + Fe -, + Al -, + organically - bound P.	occluded P by difference.
	mg P%	mg P%	mg P%
No P	228	181	47
Mod P (a)	358	309	51
(b)	315	281	34
High P (a)	388	343	55
(b)	372	334	42

(iii) Calcium -, ÷ Iron -, ÷ Aluminium - bound phosphate.

The results for the amounts of phosphate present in the sum of Ca -, ÷ Fe -, ÷ Al - bound phosphate fractions before and after phosphate addition and a comparison between the amount of phosphate added and the amount recovered in these fractions are shown in Tables I3 and I4.

From Table I3 it is evident that there was no significant change in the sum of these fractions over the I0 month experimental period (statistical analysis in Appendix). Comparison of the values obtained after I0 months storage in the presence and absence of toluene - ether supports the contention that this latter treatment had no significant effect on the amounts of phosphate occuring in the sum of these fractions.

From Table I3 it is evident that the combined Ca -, ÷ Fe -, ÷ Al - bound phosphate fraction underwent a substantial increase in the non - phosphated soils as a result of prolonged storage in the presence of moisture and toluene - ether treatment. The effect of the toluene - ether treatment is discounted as contributing to this effect because a similar increase occurred in the phosphated soils both in the presence and absence of the toluene - ether treatment. That this effect is not only a function of an extended period of storage is evidenced from the detection of a similar increase, over and above the amount of phosphate added, in the soils stored for only two months after phosphate addition (Table I4). It seems possible that the increase in inorganic phosphate noted may occur within much shorter time intervals than those investigated and that the effect arises entirely as a result of moistening the air dry soil at the commencement of the main experiment. The increase referred to above appears to be independant of the toluene - ether treatment or the phosphate addition.

Table 13. Amounts of Ca -, + Fe -, + Al - bound phosphate in the soils prior to, and at intervals following phosphate addition.

Soil	Before phosphate addition	Time interval from commencement of experiment.						
		2 mths	4 mths	6 mths	8 mths	10 mths	10 months	
		Soil Treatment						
		phosphate added in the presence of toluene - ether.					(control 2) phosphate added in absence of toluene - ether.	(control 1) No phosphate added toluene - ether treatment only
	mg P%	mg P%	mg P%	mg P%	mg P%	mg P%	mg P%	mg P%
No P	72	186	179	163	159	173	173	97
Mod P (a)	144	260	241	240	227	248	240	174
(b)	129	240	226	222	219	219	237	168
High P (a)	185	307	300	289	280	295	283	203
(b)	167	300	283	262	260	267	282	207

Table 14. Comparison between amount of phosphate added to soils and the amount recovered in the sum of the Ca -, + Fe -, + Al - bound phosphate fractions.

Soil	Amount Phosphate added.	Amount Phosphate recovered in Ca -, + Fe -, + Al - bound Phosphate fractions after 2 mths storage	Discrepancy between amount added and amount recovered
	mg P%	mg P%	mg P%
No P	89	114	+ 25
Mod P (a)	89	116	+ 27
(b)	89	111	+ 22
High P (a)	88	122	+ 34
(b)	90	133	+ 43

(iv) Organic - phosphate.

Table I5 compares the amounts of organic-phosphorus present in the soils before, and at 2 months and I0 months after, the addition of phosphate.

It is of interest to note that the Mod - P soils and High-P soils have similar amounts of organic - phosphorus whereas the No - P soil has a much reduced organic-phosphorus content This is in contrast to the findings of Saunders (I959 (b)) who found similar organic - phosphorus levels in all five of these soils regardless of the rate of phosphate fertilizer application in the field.

It is evident from Table I5 that at the two month sampling after the addition of phosphate there has been a substantial fall in the organic - phosphorus content of all five soils. This fall coincides with the increase in inorganic phosphorus discussed in the previous section and provides an explanation for the latter effect. Also the observed fall in organic - phosphorus at the two month sampling is parallelled by the behaviour of the control I samples, thereby illustrating that the drop in organic phosphorus is independent of the phosphate addition.

From Table I5, a comparison between the toluene - ether treated soils at the I0 months sampling and the corresponding control 2 samples indicates that the drop in organic phosphorus is independent of the toluene - ether treatment.

It is evident from Table I5 that there is no predictable trend in the organic phosphorus values after the initial observed decrease at the two months sampling.

Table 15. Amounts of organic - phosphate in the soils prior to, and at intervals following, phosphate addition.

Soil	Before phosphate addition	Time interval from commencement of experiment			
		2 months	10 months		
		Soil treatment			
		Phosphate added in presence of toluene - ether	phospate added in presence of toluene - ether	phosphate added in absence of toluene - ether	No phosphate added toluene - ether only.
	mg P%	mg P%	mg P%	mg P%	mg P%
No P	109	83	112	112	96
Mod P (a)	166	143	160	147	135
(b)	151	123	120	150	128
High P (a)	158	120	130	139	123
(b)	167	117	137	126	128

(v) Aluminium - bound phosphate.

The results obtained for the amounts of aluminium - bound phosphate before, and at intervals after, the addition of phosphate and the amounts of added phosphate recovered as aluminium - bound phosphate, are presented in Tables I6 and I7.

Statistical treatment of these results (Appendix) shows that there is no significant effect of time on the amounts of this form of phosphate in any of the soils examined.

From Table I6, a comparison between the soils before phosphate addition and the control I samples reveals an increase in the amounts of aluminium - bound phosphate of order of magnitude approaching that reported (Table I4) for the combined Ca -, ÷ Fe -, ÷ Al - bound phosphate fractions.

Table I7 summarises this situation. Evidently the inorganic fraction resulting from moistening of the soil samples at the commencement of the main experiment, is largely extracted by 0.5 M NH_4F at pH 8.5. The increase apparent in the aluminium - bound phosphate fraction is independent of toluene - ether treatment, or phosphate addition, and evidently occurs within the first two months after moistening of the air - dry soils; possibly early in this period.

Table 16. Amounts of Al - bound phosphate prior to, and at intervals following the addition of phosphate.

Soil	Before phosphate addition	Time interval after commencement of experiment.						
		2 mths	4 mths	6 mths	8 mths	10 mths	10 Months	
		Soil treatment						
		Phosphate added in presence of toluene - ether.					Phosphate added in absence of toluene - ether (control 2)	No phosphate added toluene - ether only (control 1)
	mg P%	mg P%	mg P%	mg P%	mg P%	mg P%	mg P%	mg P%
No P	58	163	152	144	140	152	162	71
Mod P (a)	103	222	211	202	188	209	204	127
(b)	94	198	200	186	195	184	192	116
High P (a)	148	267	271	245	248	257	238	169
(b)	125	245	246	224	219	230	224	152

Table 17. Recovery of total added phosphate in Al - bound phosphate fraction.

Soil	Actual amount phosphate added.	Amount phosphate recovered after 2 months storage	Discrepancy between amount added and amount recovered.
	mg P%	mg P%	mg P%
No P	89	105	+ 16
Mod P (a)	89	119	+ 30
(b)	89	104	+ 15
High P (a)	88	119	+ 31
(b)	90	120	+ 30

(vi) Iron - ÷ Aluminium - bound phosphate.

The results obtained for the amounts of Fe - ÷ Al - bound phosphate present in the soil before, and at intervals after, the treatments investigated are shown in Table I8. Table I9 shows the amount of phosphate recovered in the sum of the Fe - ÷ Al - bound phosphate fractions following phosphate addition, after two months of storage.

It is evident from Table I8 that there has been no significant change in the amount of the combined Fe - ÷ Al - bound phosphate fraction with time.

An increase, over and above the amount of phosphate added, is shown both for the phosphate - treated and untreated soils. As noted in the preceding section, this increase is mainly confined to the aluminium - bound phosphate fraction.

Table 18. Amounts of Fe - + Al - bound phosphate prior to, and at intervals following the addition of phosphate.

Soil	Before phosphate addition.	Time interval after commencement of experiment.						
		2 mths	4 mths	6 mths	8 mths	10 mths	10 Months.	
		Soil treatment						
		Phosphate added in presence of toluene - ether					(control 2) phosphate added in absence of toluene - ether.	(control 1) no phosphate added toluene - ether only.
	mg P%	mg P%	mg P%	mg P%	mg P%	mg P%	mg P%	mg P%
No P	58	151	149	143	139	155	147	77
Mod P (a)	117	215	220	213	189	217	213	138
(b)	103	204	213	185	195	184	209	126
High P (a)	164	268	284	250	238	283	254	185
(b)	136	234	248	224	245	214	228	145

Table 19. Recovery of total added phosphate in Fe -, + Al - bound phosphate fraction.

Soil	Actual amount phosphate added.	Amount phosphate recovered after 2 months storage.	Discrepancy between amount added and recovered
	mg P%	mg P%	mg P%
No P	89	93	+ 4
Mod P (a)	89	98	+ 9
(b)	89	101	+ 11
High P (a)	88	104	+ 16
(b)	90	98	+ 8

Summary.

Table 20 shows a comparison between the amounts of the various fractions of bound phosphate present in the soils before phosphate addition. It is evident from this Table that aluminium - bound phosphate accounts for the major portion of the total inorganic fraction of bound phosphate. However of the total phosphate present in the soils about 50% is in organically - bound form.

The main conclusions to be drawn from the experimental findings are summarised as follows:

(I) Analysis of the soils, before phosphate addition, shows that all were high in total phosphate (228 - 388 mg P%) which is distributed approximately equally between inorganic and organic forms. Most of the inorganic phosphate fraction is included in the sum of aluminium - bound, plus occluded, phosphate.

(2) There was no significant effect resulting from toluene - ether treatment of the soils, other than to qualitatively affect the microbial population estimated by plate count methods.

(3) Upon addition of phosphate and moistening of the soils at the commencement of the experiment, there was an obvious increase in inorganic phosphate over and above the amount of phosphate added, the increase was particularly evident in the aluminium - bound phosphate fraction after only two months storage.

(4) Concurrent with this increase in inorganic phosphate there was a corresponding drop in organic phosphorus.

(5) After these initial changes in the forms of phosphate binding there was no apparent change in the amounts present in each fraction over the remaining 8 months of storage.

(6) All the phosphate added at the commencement of the experiment could be accounted for in the sum of Ca - ÷ Al - ÷ Fe - ÷ organically - bound phosphates at all sampling periods, thereby indicating no transfer of phosphate to "occluded" forms.

(7) The aluminium - bound phosphate fraction was found to account for most of the added phosphate.

Table 20. Amounts of the various forms of bound phosphate in the soils investigated.

Soil	Total phosphate	Ca -, + Fe - + Al - bound phosphate (inorganic Phosphate)	organic phosphate	Al - bound Phosphate	Fe -, + Al - bound phosphate	"Occulded phosphate"
	mg P%	mg P%	mg P%	mg P%	mg P%	mg P%
No P	228	72	109	58	58	47
Mod P (a)	358	144	166	103	117	51
(b)	315	129	151	94	103	34
High P (a)	388	185	158	148	164	55
(b)	372	167	167	125	136	42

C. Truog - extractable phosphate.

The amounts of phosphate extracted by the Truog procedure are presented in Table 2I. Before phosphate addition in the laboratory, the values for Truog - phosphate were low but at the same time were noticeably higher for those soils which had received fertilizer applications in the field. The values shown for the 2 month sampling show a substantial increase for all soils After I0 months storage however it is evident that the values have tended to revert to those obtained prior to the phosphate addition. This suggests that there was a marked fall in the more "readily - available" fraction of the soil phosphate over the I0 month storage period.

Also of note is the fact that, although the amount of phosphate added to the soil at the commencement of the experiment, was approximately 90 mg P/I00g soil, only about I0 - I2 per cent of this amount was recovered by Truog - extraction of the soils after 2 months storage, and 4 - 6 per cent after I0 months storage.

Table 21 Amounts of Truog-Phosphate prior to, and at intervals following the addition of phosphate.

Soil	Before phosphate addition.	Time interval after commencement of experiment.					
		2 mths	4 mths	6 mths	8 mths	10 mths	10 mths
		Soil Treatment					
		phosphate added in presence of toluene - ether.					Phosphate added in absence of toluene - ether. (control 2)
	mg P%	mg P%	mg P%	mg P%	mg P%	mg P%	mg P%
No P	2	8	6	7	3	4	4
Mod P (a)	4	12	9	10	4	5	6
(b)	4	12	10	10	5	5	6
High P (a)	7	18	14	16	7	8	9
(b)	10	21	18	17	11	12	11

D. Reciprocal of absorption.

The results for the determination of the "reciprocal of absorption", are shown in Table 22. The values shown in brackets are the duplicates of each determination.

A comparison between the duplicates at each sampling period indicates the considerable variability obtained using this procedure. The reason for this variability cannot be ascertained at this stage, but work is proceeding in this laboratory on a range of soil types to determine the reasons for the poor repeatability of the reciprocal of absorption values obtained for the yellow - brown loams used in the current investigation.

From Table 22 it is evident that the reciprocal absorption values obtained for the soils prior to the laboratory addition of phosphate are lower than those obtained at sampling periods after the addition. Although these results suggest that the treated - soils have lost some of their phosphate retention capacity, the reduction is small, indicating the high phosphate retention characteristics of these soils. This is further illustrated by the low reciprocal of absorption values obtained for the High P soils, before laboratory addition of phosphate. The values obtained for the reciprocal of absorption at each two monthly sampling show no discernible trend over the IO month duration of the main experiment.

It may be concluded from these results that the reciprocal of absorption is unlikely to be of any great value as a method for investigating short term changes in phosphate status of soils following fertilizer application in the field.

Table 22. Reciprocal of Absorption values prior to, and at intervals following, phosphate addition.

Soil	Before phosphate addition	Time interval after the addition of phosphate.			
		2 mths	4 mths	6 mths	10 mths
No P	2.08 (2.23)	2.35 (2.63)	2.82 (2.22)	2.22 (2.35)	3.51 (2.53)
Mod P (a)	2.11 (2.19)	2.28 (2.68)	2.25 (2.30)	2.22 (2.28)	3.45 (2.83)
Mod P (b)	2.15 (2.21)	2.64 (2.76)	2.60 (2.73)	2.35 (2.53)	3.19 (3.08)
High P (a)	2.25 (2.13)	2.70 (3.18)	3.84 (3.51)	2.38 (2.41)	3.63 (3.45)
High P (b)	2.15 (2.31)	2.67 (2.73)	3.84 (3.62)	2.41 (2.46)	3.17 (2.76

E. Application of simultaneous isotopic exchange kinetics procedures.

One series of samples, that based on the No - P soil, was employed for this study. The results are shown in Tables 23,24, 25 and 26.

The anomalous results (Table 23) for the amounts of each type of exchangeable phosphate obtained at the four month sampling period result from the high ^{31}P solution value recorded in this run. Since lack of equipment prevented duplication of any one run this set of results is excluded from the following discussion.

Table 23 shows that the amounts of each type of exchangeable phosphate decreased with time and a comparison between the results obtained for the control 2 run and the ten month sampling reveals that toluene - ether treatment did not in any way affect the results obtained.

From the results in Table 24 it is evident that the amount of phosphate remaining exchangeable, two months after the phosphate addition, was approximately thirty per cent of the total amount of phosphate added to the soil. This amount decreased rapidly until at six months it was only ten per cent of the phosphate added. From six months until the end of the investigation (ten months) there was little, if any, change in the total amount of exchangeable phosphate in the soil. Corresponding to the fall in total exchangeable phosphate with time was a fall in the amount of phosphate in solution (A). Initially "A" represented about ten per cent of the total exchangeable phosphate and, although the amount of "A" fell with time, it remained a steady six to seven per cent of the total exchangeable phosphate fraction.

From Table 25 it is evident that the amount of the fast - reacting types of exchangeable phosphate (C and D) were about eighty - six per cent of total exchangeable phosphate at two months and although falling with time they still accounted for the greater portion of the total exchangeable phosphate.

The amount of the slow - reacting type (B) decreased with time, but remained a constant percentage of the total exchangeable phosphate. These general findings agree with reports by Talibudeen (1958) who showed that three-quarters of the phosphate added to a calcareous soil was recovered in the "rapidly - labile" fraction of the exchangeable phosphate, over a short term. He also found that after one month, only two - thirds of the applied phosphate was recovered in the exchangeable phosphate fraction of the soil phosphate. The analysis of the phosphate exchange reactions used by Talibudeen differs entirely from the method used in this investigation and therefore the types of exchangeable phosphate referred to by him are not necessarily the same as those reported here. Whereas Talibudeen (1958) found an increase in the slowly - exchangeable fraction of the exchangeable phosphate, at the expense of the rapidly - exchanging fraction, results of the present study (Table 23) suggest that all types of exchangeable phosphate decrease with a consequent increase in non - exchangeable phosphate.

Table 25 shows that the percentage distribution between the various types of exchangeable phosphate identified does not change appreciably over the 10 month experimental period. The fall in total exchangeable phosphate noted from Table 23 suggested that with time, most of the exchangeable phosphate was transferred to non - exchangeable forms. Since the percentage distribution (Table 25) between the various types of exchangeable phosphate does not change it appears that each type of exchangeable phosphate supplies phosphate directly to the non - exchangeable phosphate fraction. The amounts transferred in this manner are relative to the amounts of each type of exchangeable phosphate present. The various types of exchangeable phosphate presumably occur in soils due to the existence of sites which possess different energies of absorption for phosphate. (The term "absorption" is here used to describe the general removal of phosphate from solution and its binding in both exchangeable and non - exchangeabl forms).

In this discussion, the non - exchangeable phosphate is presumed to be that phosphate bound at sites which possess high energies of absorption of phosphate; types "C" and "D" are phosphate bound at sites with low energy of absorption, while "B" is that phosphate existing at sites intermediate between these two extremes.

Whether the transfer of phosphate from exchangeable forms to non - exchangeable forms occurs after phosphate has been attracted and loosely - bound to exchange sites, or by the direct absorption of phosphate from "A" onto sites which possess high energies of absorption of phosphate, cannot as yet be decided.

Table 23 shows that the method used in this investigation differentiated at least two types of exchangeable phosphate with different half - lives. One of these was a "rapidly - exchanging" type (C) with a half - life of about 1 - 2 hours, the other (B), a "slowly - exchanging" type with a half - life of 17 - 100 hours. In some instances a third type (D) was isolated but since its half - life was a fraction of an hour it is probable that this type is an artefact resulting from an unusually slow circulation time for the solution in the apparatus during ^{32}P - phosphate counting, and D is better considered, together with type C, as the total rapidly - exchanging fraction.

Talibudeen (1959) was able to discern four types of exchangeable phosphate, which he classified as "instantaneous", "rapidly - exchanging" (half - life $\frac{1}{2}$ - $1\frac{1}{2}$ hours), "medium" (half - life 2 - 8 hours) and "slow - exchanging" (half - life 25 - 40 hours). However, as previously mentioned, his analysis of the experimental results obtained from the measurement of fall in added ^{32}P - activity against time was different from the method applied in this study.

Table 25 indicates that for type B, the half - life was initially long (100 hours after 2 months storage), but that it progressively shortened until at 10 months it was only 17 hours. Similarly, the half - life of type C changed from 3 hours (at 2 months) to a steady $1\frac{1}{2}$ hours (at 4 - 10 months). It appears that type B, and type C, could be the overall measured effect which results from two or more types of exchangeable phosphate within the general identified categories. These two or more fractions of B (and C) may not be distinguishable during the experiment due to the lack of sensitivity of the instrumentation so far developed and used in this investigation. If this is the case, there may be one fraction of B (and C) with a longer half - life than is reported for B (and C) and another (or others) with a shorter half - life(s). The overall measured half - life for B (and C) would therefore depend on the relative amounts of each of these components present during the measurement of B (or C). The fall in half - life, with time, found for all the types of exchangeable phosphate (Table 25) may therefore be indicative of the loss of the slower - exchanging component from each type of exchangeable phosphate, leaving a relative abundance of the faster exchanging components. It is postulated that the loss of this slower - exchanging component of the exchangeable phosphate could in fact be the reaction:

$$Pe \longrightarrow Pf$$

(where Pe = exchangeable phosphate
P_f = non - exchangeable (fixed) phosphate.)

The actual mechanism whereby exchangeable phosphate is transposed to non - exchangeable phosphate obviously requires closer investigation.

Table 26 shows the reaction rates for the three types of exchangeable phosphate for each two - monthly sampling period. It is interesting to note that although the amount of each form of exchangeable phosphate decreased with time, the turnover, expressed as mg P/hour, of each type did not show a correspondingly large decline.

Arrambarri and Talibudeen (1959) state that there is evidence to support the contention that some of the phosphate combined with organic molecules can exchange isotopically with orthophosphate. The half - life quoted for such exchange reactions vary from a few hours to many days. In view of the high organic phosphate content of this soil (Table 15) the possibility arises that the small amount of the very slowly - exchanging component of type B, postulated above, could result from the exchange of ^{32}P - phosphate for phosphate in loose labile organic form. Also,it is interesting to note from Table 24 that the results obtained for the soil before the addition of phosphate, suggest that only one type of exchangeable phosphate (B) is present, with a half - life of six hours (Table 25). If it is conceded that at some point in the history of soil development most of the types of exchangeable phosphate (i.e. A,B,C and D) were represented, then with the subsequent passage of time, most of the very slowly - exchanging components of each type could become non - exchangeable, as a consequence of the postulation made previously. Also the rapidly - exchanging types, C and D, by virtue of the nature of their half - lives, would eventually be bound as types with the longer half - lives. The expected result after years of soil development would be the predominance of exchangeable phosphate remaining in the soils, as type B. This hypothesis explains why, after the addition of phosphate to a soil, its initial distribution between all types of exchangeable phosphate (A, B, C and D) is followed by a trend to domination of the exchangeable phosphate content of the soils by the type B with a half - life in the vicinity of 6 - 10 hours.

From the point of view of the addition of phosphate to a yellow - brown loam, in the field, it could be inferred from the results above, that initially there is likely to be a large increase in the exchangeable phosphate fraction rapidly diminishing in size after a 2 - 4 month period. Four months following application it is likely that there would be a greatly reduced supply of phosphate to the soil solution, as a consequence of the reduction in size of the "labile pool", and a probable reduction in plant availability of the applied phosphate.

Table 23. Amounts of the types of exchangeable phosphate prior to, and at intervals following addition of phosphate.

Types of exchangeable phosphate.	Before phosphate addition.	Time interval after the addition of phosphate.					
		2 mths	4 mths	6 mths	8 mths	10 mths	10 mths
		Soil Treatment.					
		Phosphate added in presence of toluene - ether.					(control 2) Phosphate added in absence of toluene - ether.
	mg P%	mg P%	mg P%	mg P%	mg P%	mg P%	mg P%
A	0.53	3.60	8.90	0.67	0.56	0.39	0.28
B	2.00	1.44	3.69	0.08	0.15	0.04	0.03
C	Nil	30.30	19.19	0.25	11.52	0.42	0.26
D	Nil	Nil	Nil	8.08	Nil	4.09	7.58
C + D	Nil	30.30	19.19	8.33	11.52	4.51	7.84
Total	2.53	35.34	31.78	9.08	12.23	4.94	8.15

Table 24. The recovery, of added phosphate, as exchangeable phosphate at intervals after phosphate addition.

Time interval after the addition of phosphate.	Amount of phosphate added.	Amount of phosphate recovered as total isotopically - exchangeable phosphate.	Amount of phosphate recovered as total exchangeable - phosphate as a percentage of the amount added.
months	mg P%	mg P%	%
2	89	35.34	39
4	89	31.78	35
6	89	9.08	10
8	89	12.23	13
10	89	4.94	6

Table 25. Percentage distribution of the types of exchangeable phosphate prior to, and at intervals following the addition of phosphate.

The respective half-lives of exchange are shown in brackets

Types of exchangeable phosphate.	Before phosphate addition.		Time interval after the addition of phosphate.											
			2 mths		4 mths		6 mths		8 mths		10 mths		10 mths	
			Soil treatment.											
			Phosphate added in presence of toluene - ether.										(control 2) Phosphate added in absence of toluene - ether.	
	%	(hrs)	%	(hrs)	%	(hrs)	%	(hrs)	%	(hrs)	%	(hrs)	%	(hrs)
A	20		10		28		7		5		8		4	
B	80	(6)	4	(99)	12	(55)	1	(36)	1	(21)	1	(17.2)	0.5	(30.3)
C + D	-	-	86	(2.8)	60	(2.6)	92	(1.2)	94	(1.4)	91	(1.4)	95.5	(1.5)
Total	100		100		100		100		100		100		100	

Table 26. The rate constants (K) for the types of exchangeable phosphate prior to, and at intervals following the addition of phosphate.

Type of exchangeable phosphate.	Rate constant	Before phosphate addition.	Time interval after the addition of phosphate.					
			2 mths	4 mths	6 mths	8 mths	10 mths	10 mths
			Soil treatment					
			Phosphate added in presence of toluene - ether.					(control 2) Phosphate added in absence of toluene - ether.
		hrs^{-1}	hrs^{-1}	hrs^{-1}	hrs^{-1}	hrs^{-1}	hrs^{-1}	hrs^{-1}
B	K_b	0.023	0.050	0.089	0.017	0.026	0.037	0.021
C	K_c	-	0.035	0.011	0.042	0.030	0.025	0.025
D	K_d	-	-	-	0.049	-	0.058	0.023

F. Total isotopically exchangeable phosphate.

From Table 27 it can be seen that there was a substantial increase in the amount of isotopically exchangeable phosphate following the addition of phosphate. After four months storage there was a rapid decrease in the size of the pool of exchangeable phosphate.

The values obtained for the total isotopically exchangeable phosphate before the laboratory addition, appear high in light of the values obtained after eight months storage. This discrepancy may have been caused by a fault in the liquid geiger tube used for ^{32}P - solution counting. It was not possible to repeat any one run, due to a restricted amount of soil available at each sampling period.

Table 27. Amounts of total isotopically exchangeable phosphate present in the soils prior to, and at intervals following phosphate addition.

Soil	Before phosphate addition.	Time interval after phosphate addition.			
		2 mths	4 mths	6 mths	8 mths
	mg P%	mg P%	mg P%	mg P%	mg P%
No P	10	115	44	23	25
Mod P (a)	85	184	35	23	31
Mod P (b)	110	180	39	16	11
High P (a)	100	200	58	45	40
High P (b)	80	239	73	32	43

IV. General Discussion.

The results of the fractionation procedure previously discussed support the finding of Saunders (1959 (b)) that phosphate applied to yellow - brown loams is recovered mainly in the iron - and aluminium - bound forms. Whereas Saunders implied that much of this phosphate was in the aluminium - bound form, the results obtained from this investigation show conclusively that aluminium - bound phosphate accounts for all of the added phosphate. The increase in apatite phosphate, reported by Saunders, following field application of phosphate, was attributed by him to the presence of residual, unreacted apatite phosphate which had been added in the superphosphate fertilizer. No change in the Calcium - bound phosphate fraction was anticipated, or found in this investigation, because the phosphate was added as a potassium salt. Volk and McLean (1963) and Kaila (1963) agree with the above finding that most of the added phosphate can be recovered in the iron - bound and aluminium - bound forms, but Fiskell and Spencer (1964) postulate the formation of occluded aluminium - bound phosphate following the addition of phosphate to soil. The formation of occluded phosphate was not evidenced in this investigation even after a 10 month period of interaction between soil and phosphate. It is therefore unlikely that occluded phosphate formation is an important factor limiting "plant - availability" of phosphate in the period normally elapsing between fertilizer applications. Fiskell and Spencer (1964) also stated that low additions of phosphate are fixed as iron - bound and aluminium - bound phosphate whilst high additions are fixed as iron - bound and calcium - bound phosphate as well as in the aluminium - bound form. This conclusion, together with that of

Laverty and McLean (1961) who state that high phosphate fixing soils fix two and one - half times as much phosphate in iron - bound forms as in aluminium - bound forms, are not supported by the results obtained in this investigation where a high addition of phosphate to a high phosphate fixing soil, resulted in an increase in the aluminium - bound phosphate fraction only.

Saunders (1959 (b)) and Burgess and Davies (1951) concluded that fertilizer application to yellow - brown loams did not result in any large accumulation of organic phosphorus. Contrary to these reports was the finding in the present investigation that the soils which had received fertilizer in the field, were higher in organic phosphorus than their unfertilized equivalent. As these soils were in fact identical with those reported on by Saunders (1959 (a), (b), and (c)) it is difficult to reconcile his organic phosphate values with those obtained in the present study. As already noted the organic phosphate values reported were determined by 2 methods including that employed by Saunders; both methods gave sensibly identical values. From Saunders data, Table 2 calculation gives C: N : organic P ratios of 100 : 7.9 : 1.0, 100 : 8.3 : 1.2, 100 : 7.4 : 1.1 for the No - P, mod - P and high - P soils respectively. Using the values obtained in this investigation for organic phosphorus, together with the Saunders' values for carbon and nitrogen these ratios now become, 100 : 7.9 : 0.8, 100 : 8.3 : 1.2, 100 : 7.4 : 1.1. Further work on a less restricted range of samples seems called for to establish definitely whether or not any build up in organic phosphorus results from fertilizer additions to yellow - brown loams.

Reference has previously been made to the decrease which occurred in organic phosphorus, with accompanying increase in inorganic forms, following moistening of the soils. This conversion of organic phosphorus to inorganic forms was presumably brought about by soil micro - organisms (even in the presence of toluene - ether treatment) and was independant of phosphate addition. It seems therefore that a fraction of the soil organic phosphorus is in a condition extremely susceptible to micro - biological attack. The increase in inorganic phosphate over and above the amount of phosphate added was found to be of the order of 30 mg P/100g soil. Using the figure quoted by Saunders (1956) for a 3 inch - acre of the New Plymouth black loam (400,000lbs) this increase is equivalent to the addition of about seven hundred weight of superphosphate. Broadbent, Jackman and McNicoll (1964) noted that drying of Egmont black loam, also an example of a yellow - brown loam, induced a pronounced stimulation in the initial rate of organic matter decomposition upon rewetting, similar to the recently documented "Birch effect". (Birch (1958)) Broadbent et al showed that the effect appeared to be due to microbial activity, and suggested that drying the allophane may alter its capacity to protect organic matter against biological attack. Evidence is insufficient, at present, for determining the importance of this release of organic phosphate to inorganic forms, following rewetting of a dry soil, as a factor contributing to plant growth in the field.

An important point which emerges from this present study is that after the initial increase in aluminium - bound phosphate following the addition of phosphate there was no tendency for the phosphate to redistribute itself among the other forms of bound phosphate. This finding contrasts with the reports of Chang and Jackson (1957(2)) and Chang and Chu (1961) who predicted that with the passage of time aluminium - bound and calcium - bound ? increase phosphates will tend to decrease with consequential use in the iron - bound phosphate fraction. Chang and Chu noted this effect after storage of a soil for 100 days at field capacity. Gotch et al (1963) reporting on the soil development of Saga polder stated that organically - bound phosphate, iron - bound phosphate and aluminium - bound phosphate increased with time, at the expense of calcium - bound phosphate. In this present investigation with yellow - brown loams the calcium - bound phosphate always represented a low and constant percentage of the total phosphate. Phosphate fractionation procedures are not designed to measure the availability of soil phosphate because they do not take into account the nature or the strength of bonding of the phosphate within the identified categories. Common experience of the performance of fertilizers on yellow - brown loams indicates that the availability of applied phosphate in these soils, as with most soils, falls with time. In the present study values obtained for Truog - extractable phosphate support the contention that although the total amounts of phosphate in each category remain constant, the availability of the phosphate changes with time. (Wild(1950)),(Cecconi (1960)), (Smith and Simpson (1950)),(Sell and Olson (1946)), (Benko et al (1963)). This apparent change in availability of the phosphate without a change in the distribution of phosphate between the various categories of bound phosphate contrasts with the report by Williams, Scott and McDonald (1958), that although aluminium-binding of phosphate is responsible for the <u>magnitude</u> of phosphate sorption by the soil, iron - binding is important for determining the strength of retention of the phosphate.

The report by Russell (1963) that the reciprocal of absorption determination gives values which correlate well with plant - available phosphate has not been borne out by the results obtained during this investigation. It is therefore doubtful at this stage whether this technique is of great importance in the study of changes in phosphate availability following phosphate additions to soil.

The suggestion of changes in the strength of binding or solubility of "fixed" forms of phosphate as shown by the decline in Truog - phosphate over the 10 month period of the present experiment is substantiated in greater detail by the results obtained with the isotopic methods.

The technique adopted for the estimation of total isotopically - exchangeable phosphate involves several modifications to the methods already proposed for the estimation of this fraction of the soil phosphate. (McAuliffe et al (1948), Gunnarsson and Fredriksson (1953), Talibudeen (1954), Barbier et al (1954), Russell (1954), Amer et al (1962)). These major modifications include the extension of the chemical and isotopic equilibrium periods and the elimination of treatments which prevent the soil/water systems from reaching true equilibrium during the isotopic exchange measurement.

The SIEK technique represents a considerable advantage over the previous attempts for analysing the total isotopically - exchangeable phosphate fraction of soil phosphate (Seatz (1954), Arrambarri and Talibudeen (1959), Weikel et al (1954), Fokin (1963)). Mathematical treatment of the results obtained using this technique enables the total exchangeable phosphate to be defined into fractions which can be characterised in amount and by half - lives of exchange. Saunders (1959 (2)) suggested that the accumulation of exchangeable phosphate following topdressing of yellow - brown loams, upset the equilibrium $P_e \rightleftharpoons P_f$ (as proposed by Wiklander (1950)), thereby causing slow fixation of added phosphate to occur in an attempt to restore an equilibrium condition. From the present study it would appear that this proposal by Saunders underestimates the rate at which phosphate fixation occurs after phosphate is added to yellow - brown loams. It appears from the evidence produced from the SIEK investigation that rapid fixation of phosphate in a non - exchangeable form occurs within the first two months after phosphate addition, because at the conclusion of the first two month period only 30% of the total added phosphate remained in an exchangeable form. The techniques employed for the isotopically - exchangeable phosphate determination reported in this investigation apply to the particular soil (New Plymouth black loam) studied and the periods necessary for establishment of chemical and isotopic equilibrium need to be determined by preliminary trials if the techniques are to be applied to other soil types or to the study of the exchange behaviour of other ionic species in soil systems.

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Summary.

A laboratory study was made of the forms in which added soluble phosphate accumulated in a yellow - brown loam (New Plymouth black loam) over a ten month period of moist storage, using the fractionation procedure of Fife (1959 a, b, 1962, and pers. comm.). Although representative of one soil type, the soil samples were selected from sites which had been subjected to different topdressing rates. Initially all the soils, regardless of the rate of fertilizer addition in the field, were relatively high in total phosphate, with approximately equal amounts in organic and inorganic forms. It was shown that a high proportion of the latter was present as aluminium - bound phosphate. The organic phosphorus content of the soil which had not received phosphate application in the field, was lower than that in the soils which had received moderate or heavy rates of fertilizer application. Phosphate added in the laboratory was completely recovered for all the soils, in the aluminium - bound phosphate fraction. After 2 months storage, the increase in aluminium - bound phosphate was found to exceed the laboratory addition of phosphate; the increase was accompanied by an equivalent decrease in organic phosphorus content. A comparable conversion of organic phosphorus to aluminium - bound phosphate was also noted with moistened control samples to which no phosphate had been added. Further, the effect was shown to be independent of the presence of a toluene-ether mixture used to limit microbial activity. No increases in iron - bound, calcium-bound or occluded phosphates resulted from the laboratory addition of phosphate. No change in the distribution of forms of phosphate in the systems was noted subsequent to the 2 - month sampling

period.

The magnitude and characteristics of the isotopically exchangeable soil phosphate fraction were investigated by application of

(a) a procedure developed by the author for the determination of the total labile pool of phosphate.

(b) the simultaneous isotopic exchange kinetics (SIEK) technique.

The exchangeable phosphate present in the soil which had received neither field nor laboratory additions of phosphate was shown to be of one type only with a half - life of exchange of approximately six hours. After phosphate addition, the total isotopically exchangeable phosphate fraction consisted of two types of exchangeable phosphate - one with a long half - life of exchange (100 hours), the other with a short half - life of exchange (3 hours). Most of the added phosphate, after 2 months storage, occurred as the shorter half - life type, i.e. rapidly exchangeable. The amount of phosphate in each type of exchangeable phosphate, and the half - life of exchange of each type, decreased with time. It was proposed that each identified type of exchangeable phosphate was composed of two (or more) components, which could not be differentiated by the equipment used. The decrease in half - life, for each type of exchangeable phosphate, was explained as the progressive loss of a slow - exchanging component of each type, leaving the relative abundance of the faster - exchanging component. It was suggested that the loss of the slow - exchanging component of each type of exchangeable phosphate accounted for the transfer of exchangeable phosphate to non - exchangeable forms which was evident over the experimental period.

Supplementary information on changes in the phosphate - supplying power of the soils during the period of storage was sought using the Truog's extraction procedure for available phosphate and Russell's reciprocal of phosphate absorption method. Truog - phosphate values increased after phosphate addition, then progressively decreased with time. The amount of phosphate extracted by this procedure was equivalent to 10 - 12 per cent of the amount added after 2 months storage, and only 5 per cent after ten months storage. The reciprocal of absorption determination was shown to be of doubtful value for reflecting the changes in soil phosphate status over the duration of the experiment.

* * *

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APPENDIX.

(a) Test for linear trend in the values obtained for calcium -, plus iron -, plus aluminium - bound phosphates.

The worked example shown below is for the values obtained for the soil Mod - P(b) which showed the most obvious trend during the experiment.

Time interval (T)	2mth	4mth	6mth	8mth	10mth
Value (V) in mgP/100g	300	284	262	260	267

Sum T = 30, Sum V = 1373

Corrected SST = $(2)^2 + (4)^2 \ldots\ldots + (10)^2 - (30)^2/5 = 40$

Corrected SSV = $(300)^2 + (284)^2 \ldots\ldots\ldots + (267)^2 - (1373)^2/5$

$= 1163$

Corrected SP (t)(V) = $(2)(300) + \ldots\ldots(10)(267) - (30)(1373)/5$

$= -180$

Part of that SS accounted for by linear regression of (V) and (T) is

$$\frac{((SP)(T)(V))^2}{SST} = \frac{(-180)^2}{40} = 810$$

therefore,

Source of variation	S.S.	df.	M.S.	F ratio	Result
Regression	810	1	810	6.9	N.S.
Residual	353	3	118		
Total	1163	4			

The results of all other sets of values also failed to reach the required ratio for significance.

(b) A similar test was applied to the values for aluminium - bound phosphate for the soil Mod - P(a) which showed the most obvious trend over the duration of the experiment. The results of this test are summarised below.

Source of variation	S.S.	df.	M.S.	F ratio	Result.
Regression	240	1	240	1.8	N.S.
Residual	389	3	130		
Total	629	4			

The results for all other sets of aluminium - bound phosphate values also failed to reach the required ratio for significance.