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The phosphorus cycle in pig slurry measured from $^{32}PO_4$ distribution rates

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SUMMARY

The rate of isotopic distribution of labelled phosphorus, added as $H_3{}^{32}PO_4$, between inorganic and organic phosphates and phosphates contained in micro-organisms was measured in pig slurry. Incorporation of ^{32}P in all these phosphates occurred quickly in both aerated and non-aerated pig slurry. On the basis of a simplified model, turnover times for phosphorus were calculated to be of the order of 10–20 weeks for both non-aerated and aerated pig slurry.

Pig slurry contains 1–2% P (of dry matter) of which 10–30% is in organic molecules and 2–3% is in micro-organisms. About 10–20% of the organic phosphates is in solution, amounting to 10–20 mg P/l. The concentration of inorganic P in solution is of the order of 10–100 mg/l though, at low Ca/P ratios in the feed, can be as high as 1000 mg/l.

Organic phosphates in solution in pig slurry are of high molecular weight and probably consist of DNA complexes with polyphosphates, Ca and (if used in the feed) Cu.

It is concluded that all organic phosphates in pig slurry are of microbial origin and that the feed composition has little influence on the organic phosphate content of the slurry.

Arguments for application of the results to pig slurry in general and to wastes from other animals are given.

INTRODUCTION

Phosphorus in animal wastes, which in some agricultural areas are applied to the soil in excessive amounts, is a potential pollutant of surface and ground water. Inorganic phosphate is usually assumed to be effectively adsorbed by the soil, though excessive application of solutions of moderate phosphorus concentration can be shown to give a rapid movement of inorganic phosphate in the soil (Goodrich, 1970). The predominance of organic phosphates in phosphorus movement in the soil has been noted by Hannapel, Fuller & Bosma (1963), Hannapel, Fuller & Fox (1963), Rolston, Rauschkolb & Hoffmann (1975), and Campbell & Racz (1975).

In pig slurry 10–30% of total phosphorus (P) is contained in organic phosphates, of which a small part is present as inositolhexaphosphate and the rest is of unknown composition, though probably of microbial origin (Sauerlandt, 1960; Peperzak *et al.* 1959; Caldwell & Black, 1958; McAuliffe & Peech, 1949).

From solubility data of calcium and phosphorus (Barrow, 1975) it can be concluded that inorganic phosphate in pig slurry is present as calcium hydrogen phosphate ($CaHPO_4.2H_2O$) and calcium phosphates of lower solubility of very small particle diameter (Larsen, 1968).

As part of a thorough study of the movement of organic phosphates from manure in the soil, we considered it important to establish the origin and nature of these phosphates by elucidating the phosphorus cycle in pig slurry during aerobic and anaerobic storage. This paper reports an attempt to obtain information on this cycle by adding radioactive phosphorus as $H_3{}^{32}PO_4$ to pig slurry and studying the distribution rate of this labelled phosphorus between inorganic and organic phosphates in the solid phase, the micro-organisms and the liquid phase as a function of time and type of storage. In this way it is also possible to estimate the relative contribution of dietary and microbial phosphates to the total amount of organic phosphate in pig slurry.

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MATERIALS AND METHODS

Two portions of 10 l of slurry were made by mixing pig faeces and urine in such proportions that a mixture with a dry matter content of 7 % was obtained. To each of these batches 0·45 mCi of ^{32}P as H_3PO_4 was added. One of these radioactive slurries was aerated and the other was left undisturbed, except when sampling. The temperature was kept between 20 and 25 °C. Both slurries were sampled 1 day, 2 weeks, 6, 9 and 15 weeks after the addition of ^{32}P. In the 1 l samples pH, dry matter, total and inorganic phosphorus were determined. Radioactivity of the dried samples was determined after destruction with HNO_3/H_2SO_4 (1 : 1 by volume). Activity was measured directly (Philips liquid scintillation analyser), making use of ^{32}P Cerenkov radiation. The counting efficiency was about 40 %.

Part of the sample was centrifuged, first at 2000 *g*, then at 40000 *g* followed by filtration through a series of membrane filters down to a pore diameter of 0·2 μm. The slurry liquid passing the 0·2 μm filter was subjected to gel filtration through a column of Sephadex G-100 (Pharmacia), using double distilled water as elutant. The eluate of the gel column was divided into three fractions. The first fraction contained compounds of high molecular weight, the second and third fractions contained compounds of increasingly lower molecular weight. Radioactivity in these fractions was again measured after destruction.

Phosphorus in samples and fractions was determined colorimetrically using ascorbic acid (Murphy & Riley, 1962) or metol (= 4-methyl amino phenol sulphate) (Rameau & Ten Have, 1950) as reducing agent. The metol method was found to be the least subject to disturbance, but the Murphy & Riley method was more sensitive by a factor 10 and was therefore used at low phosphate concentrations.

Inorganic phosphate in dried slurry was determined by extracting it with a solution containing 20 ml HF (40 %), 400 ml HCl (36 %), and 18 ml $TiCl_4$ made up to 1 l (Tinsley & Özsavasci, 1975). Two grams of dried slurry were refluxed with 50 ml of this extractant for 5 min on a boiling water bath, cooled quickly, filtered (glassfilter G3) and washed. Phosphorus in the filtrate was measured directly by the metol method and equated to the inorganic phosphorus in the pig slurry.

Inorganic phosphorus in dried slurry was also determined in a more subtle way by extracting first with 10 % TCA (trichloroacetic acid) and then with 0·01 M EDTA (disodium salt of ethylene diamine tetraacetic acid) in 0·1 N-NaOH. Between 1 and 2 g of dried slurry were shaken with 100 ml TCA solution for 3 h at 0 °C, centrifuged and the residue washed with water. The residue was then shaken with 100 ml NaOH/EDTA solution for 12 h, centrifuged and washed with the extractant. Both extracts were analysed for inorganic phosphorus directly and for total phosphorus after evaporation of water and subsequent destruction. It was found that in this way all phosphorus compounds could be extracted and that the quantities of total and inorganic phosphorus were similar to the quantities found by extracting with $HF/HCl/TiCl_4$.

Slurry was dried in vacuum at 60 °C and also freeze-dried. No significant difference in results of inorganic and organic P analyses was found between these two methods of drying. The dry-matter content of the pig slurry was determined from the loss of weight at 105 °C after 12 h.

The difference between total and inorganic phosphorus is termed organic phosphorus. It is thus possible that in this way polyphosphates, which can occur in micro-organisms and solution (Hooper, 1973; Fuhs & Chen, 1975) are partly measured as organic phosphates. The term organic phosphates is thus used for both phosphate contained in organic molecules and for stable polyphosphates. As microbial polyphosphates often occur complexed with proteins, DNA and RNA (O'Kelley, 1973) this is not inappropriate.

ISOTOPIC DISTRIBUTION

The radioactivity in the slurry liquid, corrected for radioactive decay, decreases due to isotopic distribution between inorganic and organic phosphate compounds in the solid and liquid phase and in micro-organisms. Uptake of inorganic phosphate from solution by bacteria is a rapid process (Hayes & Phillips, 1958; Hodson, 1973) as is exchange with solid mineral phosphates (Pomeroy, Smith & Grant, 1965). In Fig. 1 the various pathways involved in the distribution of ^{32}P in pig slurry are shown. In kinetic terms this model can be approximated as follows:

$$^{32}P'_m \underset{k}{\overset{\leftarrow}{\rightarrow}} {}^{32}P'_s \qquad (1)$$

in which

$^{32}P'_m$ = specific activity in solution,

$^{32}P'_s$ = specific activity in solids.

If $^{32}P_m$ and $^{32}P_s$ are the respective activities (counts per minute) in solids of a given volume of slurry and n_m and n_s are the corresponding amounts (mg) of total P, then the following relations hold:

$$^{32}P'_m = \frac{^{32}P_m}{n_m},$$

$^{32}P'_{mo} = {}^{32}P_o/n_m$ = specific activity in solution at t(ime) = 0, and from this:

$$^{32}P'_s = \frac{^{32}P_o - n_m \cdot {}^{32}P'_m}{n_s},$$

in the experiment $^{32}P_o$ = 35000 cpm (counts per minute)/ml.

At equilibrium $^{32}P'_m = {}^{32}P'_s$ and before equilibrium is attained

$$\frac{\delta {}^{32}P'_m}{\delta t} = k({}^{32}P'_s - {}^{32}P'_m)$$

or, substituting for $^{32}P'_s$,

$$\frac{\delta {}^{32}P'_m}{\delta t} = \frac{k.{}^{32}P_o}{n_s} - \frac{1+\kappa}{\kappa}.k.{}^{32}P'_m,$$

where

$$\kappa = n_s/n_m.$$

Integrating gives

$$\ln ({}^{32}P'_{mt} - {}^{32}P'_{m\infty}) = -A.t + B, \qquad (2)$$

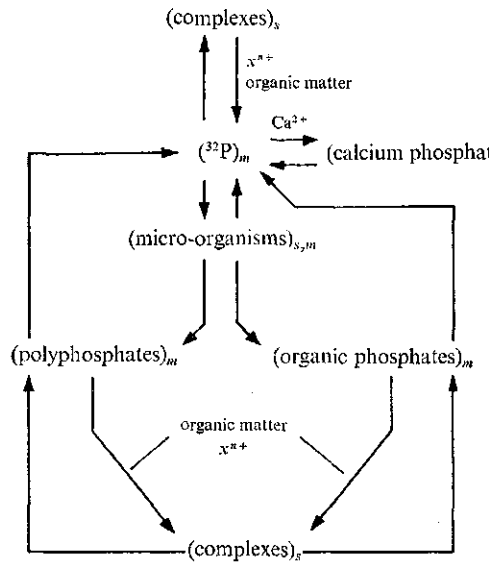

Fig. 1. Model for the phosphorus cycle in pig slurry, according to which labelled phosphorus (^{32}P) is distributed. s = solids; m = in solution.

in which

$$A = \frac{1+\kappa}{\kappa}.k, \quad \text{and} \quad B = \ln \{{}^{32}P'_{mo} - {}^{32}P'_{mo}/(1+\kappa)\}.$$

The radioactivity in solution at equilibrium is calculated from:

$${}^{32}P'_{m\infty} = {}^{32}P'_{mo}/(1+\kappa). \qquad (3)$$

The parameter κ is the capacity ratio of phosphorus in the slurry and is taken as the ratio between total phosphorus in solids and micro-organisms ($=n_s$) and total phosphorus in solution ($=n_m$), both in a unit volume of slurry. The labelled phosphorus is thus assumed to become distributed between all phosphate fractions. If this is not the case the capacity ratio of phosphorus must be used for only those phosphate fractions between which ^{32}P is distributed.

RESULTS

Distribution of ^{32}P between solid and dissolved phosphates

Results of chemical analyses of the slurries at various times are given in Tables 1 and 2. Total organic P content is slightly higher in the case of aerated slurry, while inorganic P in solution is significantly lower. The total amount of organic phosphorus can be said to remain constant for both aerated and non-aerated slurry during the time of storage (3 months).

From the data in Tables 1 and 2 the capacity ratios, as used in equation (3) can be calculated for total, inorganic and organic phosphorus in pig slurry. Results are given in Table 3. The specific activity of phosphorus in thoroughly mixed slurry samples was checked at each sampling. The average value from four samplings was 33000 cpm/mg P for total phosphorus and 40000 for inorganic phosphorus. The coefficient of variation of these values was 3–4 %.

In Table 4 the activities in slurry liquid, obtained after filtration through a 0·2 μm membrane filter,

Table 1. *Inorganic and organic phosphorus content of non-aerated pig slurry*

Sampled after	D.M. (%)	P_t	P_{in} (% of D.M.)	P_{org}	pH	P_t < 0·2 (mg/l slurry liquid)	P_{in} < 0·2 (mg/l slurry liquid)	P_{org} < 0·2 (mg/l slurry liquid)
1 day	6·8	1·48	1·26	0·2	8·4	105	74	30
2 weeks	6·7	1·53	1·31	0·2	8·3	135	90	45
6 weeks	6·3	1·62	1·35	0·3	8·6	161	148	10
9 weeks	6·1	1·64	1·39	0·3	8·7	144	127	20
15 weeks	5·6	1·80	1·60	0·2	8·5	110	—	—
S.D.	0·06	0·032	0·028	0·07	—	2·6	2·2	5

P_t, P_{in} and P_{org} = total, inorganic and organic phosphorus (as P) respectively in dried pig slurry; < 0·2 = in solution after filtration through a 0·2 μm membrane filter (= 'slurry liquid'); Ca/P ratio in the feed used was 1·2–1·5.

Table 2. *Inorganic and organic phosphorus content of aerated pig slurry*

Sampled after	D.M. (%)	P_t	P_{in} (% of D.M.)	P_{org}	pH	P_t < 0·2 (mg/l slurry liquid)	P_{in} < 0·2 (mg/l slurry liquid)	P_{org} < 0·2 (mg/l slurry liquid)
1 day	6·6	1·55	1·25	0·3	8·4	105	57	50
2 weeks	6·0	1·70	1·35	0·4	9·0	100	74	30
6 weeks	5·2	1·95	1·60	0·4	9·0	61	43	20
9 weeks	4·1	2·50	2·00	0·5	8·9	57	35	20
15 weeks	—	—	—	—	—	55	—	—
S.D.	0·05	0·038	0·031	0·08	—	1·5	1·0	3

P_t, P_{in} and P_{org} = total, inorganic and organic phosphorus (as P) respectively in dried pig slurry; < 0·2 = in solution after filtration through a 0·2 μm membrane filter (= 'slurry liquid'); Ca/P ratio in the feed used was 1·2–1·5.

Table 3. *Capacity ratios for total, inorganic and organic phosphorus in pig slurry, calculated from Tables 1 and 2*

Sampled after	κ_t (non-aerated slurry)	κ_{in} (non-aerated slurry)	κ_{org} (non-aerated slurry)	κ_t (aerated slurry)	κ_{in} (aerated slurry)	κ_{org} (aerated slurry)
1 day	9·3	11·4	4·2	9·6	14·6	3·6
2 weeks	7·1	9·4	2·5	9·9	10·5	7·6
6 weeks	5·8	5·1	13·0	16·5	19·4	9·6
9 weeks	6·4	6·1	8·5	17·8	23·4	8·7
15 weeks	8·7	—	—	—	—	—

κ_t, κ_{in}, κ_{org} are the capacity ratios for total, inorganic and organic phosphorus respectively = the ratios between phosphorus in solids (including micro-organisms) and dissolved phosphorus.

are given for the various samples. For aerated slurry Table 4 shows that the slurry liquid has a specific activity approaching the equilibrium value for distribution between all phosphates in about 15 weeks. In the non-aerated slurry the distribution is still far from equilibrium after 15 weeks, the specific activity being much higher than the equilibrium value of 33 000 cpm/mg P for distribution between total phosphorus or 40 000 for distribution between inorganic phosphorus only. To calculate the kinetic constant k (equation 1) the specific activities for total phosphorus from Table 4 were plotted according to equation (2), as shown in Fig. 2. For aerated slurry k = 0·5/week and for non-aerated slurry k = 0·07/week, giving times for complete turn-over of phosphorus of 2 and about 15 weeks respectively. These turn-over times are of course for the entire process of exchange, without giving information on the separate contributions of calcium phosphates, micro-organisms, organic phosphates, etc.

The higher apparent turn-over rate measured for aerated slurry is primarily caused by rapid exchange with calcium phosphates, due to continuous mixing.

Distribution of ^{32}P between dissolved organic and inorganic phosphates

By subjecting the slurry liquid after filtering through a 0·2 μm membrane filter to gel filtration, organic phosphates can be separated from inorganic phosphates. Results are shown in Fig. 3. All inorganic phosphates (and also coloured compounds) are eluted between 45 and 85 ml (not shown in Fig. 3). Thus using Sephadex G-100 an almost complete separation between organic and inorganic phosphates in slurry liquid can be obtained. Furthermore, it can be concluded that phosphates eluting between 20 and 45 ml must be of high molecular weight (of the order of 10^6 and higher). Tests for DNA (Kaká$\check{c}$ & Vedjdělek, 1974, pp. 872–3, 922–3) were positive but not conclusive. The first fraction was also turbid, caused by particles much smaller than 0·1 μm. Treatment of the first fraction with HCl to 5 N and subsequent boiling for 5 min resulted in a decrease of organic phosphorus of 20 %, but the turbidity remained. It is possible that the turbidity is caused by aggregates of DNA, Cu (the concentration of which in slurry liquid is about 1 mg/l), Ca and (poly) phosphates (Scharpf, 1973; O'Kelley, 1973).

In Table 5 the activities and specific activities are given for slurry liquid fractions obtained by gel filtration on Sephadex G-100. From Table 5 it follows that in slurry liquid an even distribution of ^{32}P between inorganic (eluate fraction 55–85 ml) and organic phosphates (eluate fraction 20–45 ml) is approached in aerated slurry more quickly than in non-aerated slurry. The rate constant for turnover of dissolved phosphorus can be calculated from

$$\frac{\delta^{32}P'_{org}}{\delta t} = k.(^{32}P'_{in} - ^{32}P'_{org}) \qquad (4)$$

in which k = rate constant, t = time, $^{32}P'_{org}$ = specific activity in gel filtrate fraction 20–45 ml,

Table 4. *Activities in pig slurry filtered through a 0·2 μm membrane filter*

	Non-aerated			Aerated		
Sampled after	Activity (cpm/ml)	Specific activity total P (cpm/mg)	Specific activity inorg. P (cpm/mg)	Activity (cpm/ml)	Specific activity total P (cpm/mg)	Specific activity inorg. P (cpm/mg)
1 day	22600	215000	300000	18600	177000	326000
2 weeks	16500	122000	183000	11500	115000	155000
6 weeks	14000	87000	94500	2300	37700	53500
9 weeks	12000	83000	94500	2000	35000	60000
12 weeks	10500	—	—	1900	—	—
15 weeks	6200	56000	—	1800	33000	—
c.v. (%)	1	3	3	2	4	4

The activities are corrected for radioactive decay to 0 days. Counting time was 20 min in a volume of 20 ml.

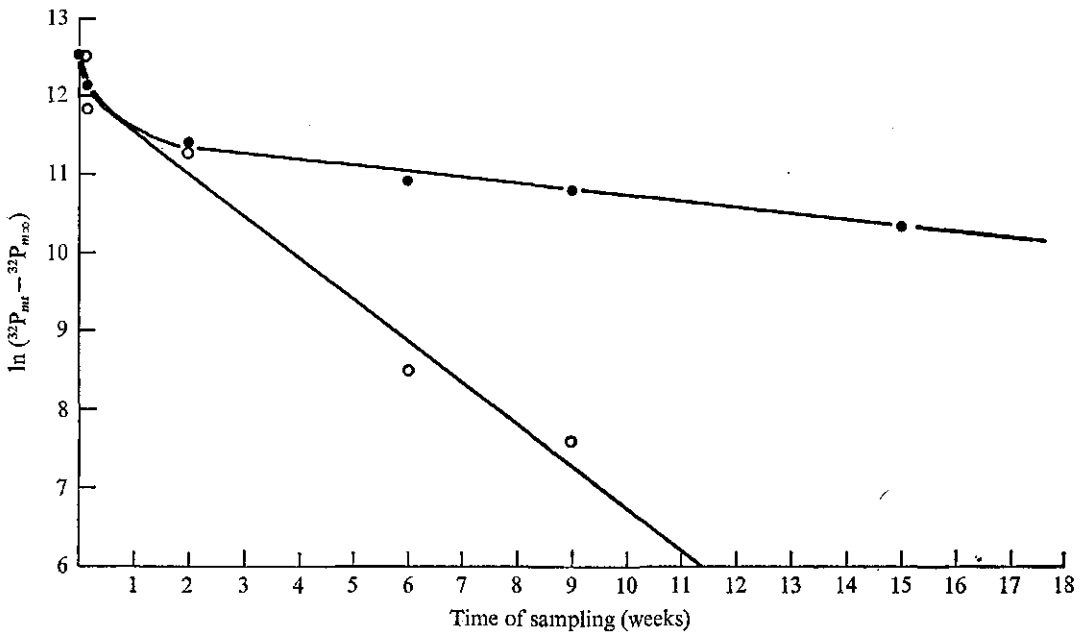

Fig. 2. Plot of the radioactivity in the slurry liquid filtered through a 0·2 μm membrane filter as a function of time, according to equation 2, for aerated (○) and non-aerated (●) slurry.

$^{32}P'_{in}$ = specific activity in gel filtrate fraction 55–85 ml.

Applying equation (4) to the data in Table 5 gives a k value of 0·05–0·1/week for both aerated and non-aerated slurry. It can thus be said that the time for complete turn-over of phosphorus in pig slurry (at 20–25 °C), based on reaction rates, calculated for dissolved phosphorus, lies between 10 and 20 weeks for both aerated and non-aerated slurry. This turn-over time is determined by biological uptake and release of dissolved inorganic and organic phosphates and not by exchange with mineral (Ca) phosphates.

Phosphate in micro-organisms

From the number of microbial cells/ml slurry, 10^8–10^{10} (Erickson, Ellis & Tiedje, 1975), the phosphorus content and weight of a cell, 0·5–2 % and 10^{-12} g respectively (Schlegel, 1972, pp. 18–19), a possible range of 0·0005–0·2 mg P present in microbes/ml slurry can be calculated.

In order to get a better estimate of the amount

of radioactivity and phosphorus incorporated in micro-organisms, after 12·5 weeks an aliquot of thoroughly mixed slurry was treated with chloroform (10 % by volume) and left for 24 h at about 20 °C. Another aliquot was treated in the same way, but after adding some sand it was shaken at 35 °C for 24 h. The samples were then centrifuged (40000 *g*) for 1 h, after which the radioactivity was measured in the supernatant. Reference samples were treated in the same way without adding chloroform. The increase in radioactivity (counts corrected for radioactive decay) varied from 50 to 250 cpm for aerated slurry and from 700 to 1000 cpm for non-aerated slurry (three measurements).

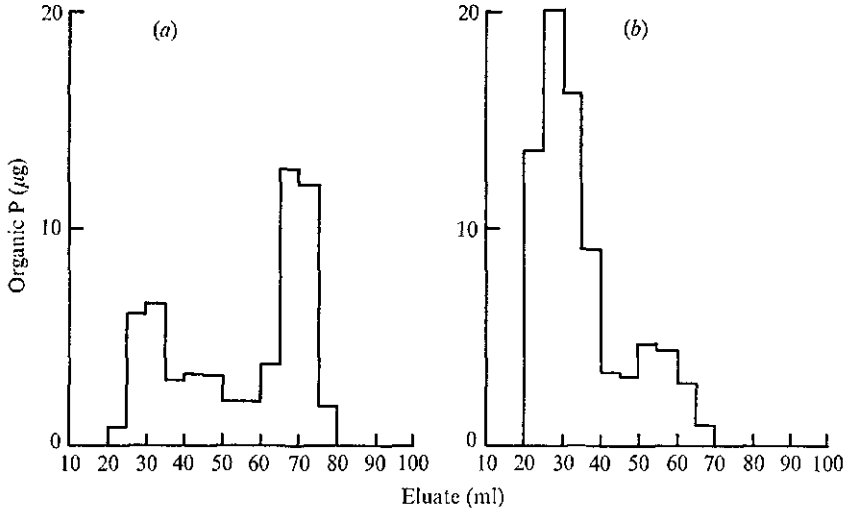

Fig. 3. (*a*) Gelfiltration of organic phosphate in slurry liquid filtered through a 0·2 μm membrane filter through a column of Sepharose 2B (Pharmacia). Organic phosphorus was measured as the difference between total and inorganic phosphorus in the fractions. Total volume of the column = 70 ml, elution volume for blue dextran = 25 ml. The exclusion limit for Sepharose 2B is given as 10^7–10^8 (molecular weight). (*b*) Gelfiltration through a column of Sephadex G-100 (Pharmacia). Total volume of column = 62 ml, elution volume of blue dextran = 15 ml. The exclusion limit for Sephadex G-100 is given as 10^5–10^6 (molecular weight).

Table 5. *Activities (cpm/ml) and specific activities (cpm/mg P) in fractions obtained by gelfiltration of pig slurry through Sephadex G-100, after filtration through a 0·2 μm membrane filter*

Activity			Specific activity		
Non-aerated slurry	Aerated slurry	Eluted volume (ml)	Non-aerated slurry	Aerated slurry	Sampled after
20	160	20–45	500	4600	1 day
400	420	—	10000	9000	2 weeks
2500	1200	—	30000	18000	6 weeks
5000	1300	—	50000	21000	9 weeks
370	330	45–55	30000	27500	1 day
750	220	—	25000	22000	2 weeks
11400	720	—	85000	28000	6 weeks
10000	1200	—	80000	46000	9 weeks
108000	95000	55–85	215000	190000	1 day
81000	55000	—	133000	126000	2 weeks
57000	8700	—	95000	43000	6 weeks
45000	7000	—	82500	35000	9 weeks

The coefficient of variation varies from 10 % at low activity (20 cpm/ml) to 1 % at the highest activities; the corresponding coefficients of variation in the specific activity are 15 and 5 %. The activities are corrected for radioactive decay to 0 days. Counting time was 20 min in a volume of 20 ml.

Table 6. *Results of radioactivity measurements in pig slurry after removal of calcium by extracting twice with $NaHCO_3$/EDTA solution at ph 8·5, followed by lysis of micro-organisms and a third extraction. Reference samples were treated in the same way without lysis. The sample was taken 14 weeks after addition of ^{32}P*

	Non-aerated slurry				Aerated slurry			
	Activity, no lysis (cpm/ml)	P_{tot} (mg/l)	Activity, lysis (cpm/ml)	P_{tot} (mg/l)	Activity, no lysis (cpm/ml)	P_{tot} (mg/l)	Activity, lysis (cpm/ml)	P_{tot} (mg/l)
Supernatant	10000	—	10000	—	3500	—	3500	—
1st extract	20000	—	20000	—	20000	—	20000	—
2nd extract	13000	—	13000	—	10000	—	10000	—
3rd extract	5500	270	7000	300	4500	140	5500	170
c.v. (%)	5	2	5	2	10	2	10	2

The data are averages of four measurements. Lysis with chloroform or by a freezing (−35 °C) and thawing cycle gave identical results.

Part of the phosphorus released after cell lysis by chloroform could precipitate with calcium and thus not contribute to the increase of radioactivity of the solution. To overcome this problem another procedure was followed. Pig slurry was centrifuged at 6000 *g* and the supernatant decanted and replaced by a solution of pH = 8·5, containing 1 % (by weight) $NaHCO_3$ and 0·05 M-EDTA, then shaken for 15 min, centrifuged and decanted again. This procedure was repeated. In this way calcium is extracted from the slurry, while micro-organisms remain in the residue. After decanting a third time chloroform was added to the residue or the residue was subjected to a cycle of freezing and thawing to lyse micro-organisms. The residue was then shaken with $NaHCO_3$/EDTA solution and centrifuged once more, after which radioactivity and total phosphorus content of the supernatant were determined. Reference samples were treated in the same way without lysing the micro-organisms. Results are given in Table 6, from which it can be seen that about 0·03 mg P is present in microbes/ml slurry. This represents about 15–20 % of total organic P in pig slurry.

Total organic phosphate

Vacuum-dried slurry was extracted, as described under Materials and Methods, with TCA and NaOH/EDTA, after which the specific activities in the extracts were determined. The results for the various sampling dates are given in Table 7. With TCA about 50 % of all organic phosphorus in pig slurry and practically all inorganic phosphorus is extracted. With NaOH/EDTA the remaining organic phosphorus is extracted. This extract thus contains practically only organic phosphorus. In Table 4 the specific activity in the aerated slurry liquid is seen to approach the equilibrium value of 33000 cpm/mg P for distribution between all phosphates, which means that all organic and inorganic

Table 7. *Specific activities (cpm/mg P) in extracts of dried pig slurry at various times of sampling*

	Extraction with TCA		Extraction with NaOH/EDTA	
Sampled after	Non-aerated	Aerated	Non-aerated	Aerated
1 day	36500	36500	29000	18000
6 weeks	34000	32000	66500	39000
9 weeks	34000	32000	52500	36500
c.v. (%)	5	5	5	5

phosphates take part in the cycle of Fig. 1. This is corroborated to a certain extent by the data in Table 7 for the NaOH/EDTA extracts. For non-aerated slurry the data point to a similar distribution, but the time necessary to obtain complete equilibrium conditions is much longer.

DISCUSSION

From the data obtained, it can be concluded that phosphorus in organic and inorganic phosphate compounds in pig slurry takes part in a biological reaction cycle with a turn-over time of 10–20 weeks. Turn-over rates in aerated and non-aerated slurry are not significantly different. Organic phosphorus content of aerated pig slurry is only slightly higher than that of non-aerated slurry. The driving force in the phosphorus cycle is provided by micro-organisms; 10–20 % of all organic phosphorus in the slurry is contained in microbial cells, another 10–20 % is in solution and the rest is in solids. Fresh manure from young animals and poultry can have high organic P contents (up to 60 % of total P), mainly due to organic phosphorus from the feed (Peperzak *et al.* 1959; Sauerlandt, 1960). Because of

Table 8. *Influence of the phosphorus composition of pig feed on the phosphorus composition of urine and faeces Separate samples were taken from groups of three pigs fed the same diet. The weight of the pigs was about 68 kg*

Ca/P ratio	In feed (% of D.M.)			In faeces (% of D.M.)			In urine (mg/l)			In urine* (mg/l)		
in feed	P_t	P_{in}	P_{org}	P_t	P_{in}	P_{org}	P_t	P_{in}	P_{org}	P_t	P_{in}	P_{org}
0·46	0·32	0·05	0·27	1·35	1·05	0·3	44	31	13	11	5	—
0·22	0·59	0·06	0·53	1·40	1·18	0·2	1090	1000	90	870	800	6
0·30	0·50	0·17	0·33	1·53	1·22	0·3	650	600	50	520	480	40
0·37	0·62	0·08	0·54	1·40	1·18	0·2	870	800	70	700	660	40
0·55	0·60	0·08	0·52	1·57	1·31	0·3	650	600	50	520	500	20
					c.v. (%)							
3	2	4	3	2	2	20	5	5	100	2	2	50

Dry matter in faeces was 30 % (S.D. = 2·0) for all samples, for urine this was 1·5 % (S.D. = 0·50).
* Urine filtered through a 0·2 μm membrane filter.

the phosphorus cycle, the organic phosphorus content of the feed can be expected to have little influence on the organic phosphorus in manure, stored a long time or from animals where decomposition and resynthesis of organic phosphates in the digestive tract occurs fast (McAuliffe & Peech, 1949).

To investigate the influence of the feed in the case of pigs, faeces and urine were analysed from five groups of three animals given feeds of various Ca, inorganic and organic P contents. From the data (shown in Table 8) it can be seen that organic P content of faeces is fairly constant and is not influenced by the phosphate composition of the feed, while inorganic P content tends to rise with increasing total P content of the feed. Similar results have been mentioned in the literature (Bromfield, 1961) for sheep faeces. It can be concluded from Table 8 that faeces contributes most to organic phosphorus in slurry. Inorganic phosphorus in urine can be very high, apparently at a low Ca/P ratio. The organic P content of pig slurry, ranging between 15 and 25 % of total P and between 0·2 and 0·4 % of D.M., can be said to be an equilibrium value in the phosphorus cycle. Data from the literature (Peperzak *et al.* 1959; Sauerlandt, 1960) and our own work show that for other animals organic P content of manure, on storage, approaches a similar equilibrium value, pointing to the same phosphorus equilibrium or cycle as in pig slurry.

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