Comparison of radioisotope methods for the measurement of phosphate absorption in normal subjects and in patients with chronic renal failure

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Summary

1. Intestinal phosphate absorption was measured in normal subjects, in patients with chronic renal failure, and in post-transplant patients, by a double isotope technique involving oral administration of $^{32}$P and simultaneous intravenous injection of $^{33}$P with subsequent deconvolution analysis.

2. By this technique intestinal phosphate absorption has been shown to have two components: an initial rapid phase, which is completed by 3 h, and a slower more prolonged phase, which continues beyond 7½ h.

3. Phosphate malabsorption has been demonstrated in chronic renal failure and transplant patients, which is accounted for by impairment of the initial rapid phase of absorption.

4. Results obtained by deconvolution analysis have been compared with other estimates of phosphate absorption obtained from analysis of $^{32}$P radioactivity curves alone.

5. The fractional hourly rate of absorption and the plasma $^{32}$P radioactivity at 60 min corrected for extracellular fluid volume provided the best approximations to the result obtained by deconvolution analysis, with respect to both the maximal rate of phosphate absorption and cumulative percentage phosphate absorption.

Key words: chronic renal failure, deconvolution analysis, intestinal phosphate absorption, post-renal transplant.

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Introduction

Because of the limitations and the intrinsic errors of traditional balance studies, recent work in the field of intestinal phosphate absorption in man has been centred on the use of radiophosphorus ($^{32}$P) (Canniggia & Gennari, 1971; Marshall, 1976; Farrington, Epstein, Varghese, Newman, Moorhead & Sherlock, 1979a; Farrington, Varghese, Newman, Ahmed, Fernando & Moorhead, 1979b). The essence of these methods is that an estimate of phosphate absorption is obtained from the analysis of one or more plasma levels of $^{32}$P after the administration of an oral dose. Such single isotope methods tend to provide estimates of the initial rate of phosphate absorption, which may be related to total phosphate absorption (Marshall, 1976). This approach, however, fails to take into account a number of important factors which also influence the blood levels of $^{32}$P, such as the rate of tissue uptake of $^{32}$P and the rate of its renal excretion.

In the study of intestinal calcium absorption alternative radioisotope techniques are available such as whole-body radioactivity counting methods (Whelton, Kehayoglou, Agnew, Turnberg & Sherlock, 1971), or techniques involving the simultaneous oral and intravenous administration of different isotopes of calcium, with subsequent deconvolution analysis of the plasma levels of these isotopes (Hart & Spencer, 1967). Such methods have been used to validate the methods based on the use of single isotopes. In the case of intestinal phosphate absorption whole-body counting is not possible because of the lack of a gamma-emitting P isotope, and though this method has been applied utilizing the
Bremsstrahlung emission of $^{32}\text{P}$ (Cabrejas, Mendez Falcon, Morgenstern, Degrossi & Mautalen, 1977), the doses of the isotope necessary were prohibitively large for a general application.

This study describes the adaptation of a dual isotope method (Hart & Spencer, 1967), developed for the assessment of calcium absorption, to the problem of intestinal phosphate absorption by using orally administered $^{32}\text{P}$ and the simultaneous intravenous administration of $^{33}\text{P}$. This method permits the calculation of both cumulative phosphate absorption (Hart & Spencer, 1967) and the maximal rate of phosphate absorption (adapted from Reeve, Hesp & Veall, 1974). The parameters have been compared with estimates of phosphate absorption derived simultaneously by analysis of one or more blood levels of $^{32}\text{P}$ alone. By such means it was hoped to select the best approximations to both the maximal rate of phosphate absorption and cumulative phosphate absorption from parameters which could be derived from data obtainable from a single isotope method.

**Methods**

**Subjects**

Informed consent was obtained from all subjects before the study. There were three groups of subjects: one group consisted of 14 normal controls, another consisted of 10 patients with chronic renal failure (their mean creatinine clearance being $6 \pm 4 \text{ ml/min}$) and the third group consisted of six patients, 1-36 months after renal transplantation (their mean creatinine clearance being $48 \pm 14 \text{ ml/min}$). The mean age of all subjects was $37 \pm 13 \text{ years}$ and there was no significant difference between the groups with respect to age. None of the normal controls was receiving any drugs. Five of the chronic renal failure patients were taking aluminium hydroxide, which was stopped 24 h before the test. All of the transplant patients were taking standard immunosuppressive agents, prednisolone (10-60 mg daily) and azathioprine (50-100 mg daily). In addition, one was taking cyclosporin A (15 mg/kg). Four of the transplant patients were taking 1-$\alpha$-hydroxycholecalciferol (0.5-1 $\mu$g/day) and one of them had previously undergone parathyroidectomy.

**Materials**

$^{32}\text{P}$ for oral administration was obtained as the orthophosphate from The Radiochemical Centre (Amersham, Bucks, U.K.). $^{33}\text{P}$ also as the orthophosphate, was supplied in sterile form by New England Nuclear (Winchester, U.K.). Before intravenous injection the $^{33}\text{P}$ solution was passed through a 0.22 $\mu$m Millipore filter and equal portions were measured into 2 ml flame-sealed ampoules. Random checks of the contents of these ampoules indicated that the $^{33}\text{P}$ solution was sterile and pyrogen-free.

**Phosphate absorption tests**

The subjects were fasting from the previous midnight and the tests were carried out commencing at approximately 09.00 hours. Subjects remained fasting for the first 3 h of the test. An oral dose of 5 $\mu$Ci of $^{32}\text{P}$ with 50 mg of phosphorus carrier as sodium orthophosphate in 250 ml of dilute orange squash was administered at time zero and a simultaneous intravenous injection of 5 $\mu$Ci of $^{33}\text{P}$ was given. Samples of blood (10 ml) were collected at 0, 15, 30, 60, 90, 120, 180, 240, 360 and 450 min and separated immediately. The activity of $^{32}\text{P}$ and $^{33}\text{P}$ in each plasma sample was assessed by liquid scintillation counting with a Wallac scintillation counter by a method previously described for simultaneous counting of $^{45}\text{Ca}$ and $^{32}\text{P}$ (Farrington et al., 1979a). Because of the low count rates in these samples it was necessary to count for periods up to 1 h, and about 5000 counts were collected per sample for both $^{32}\text{P}$ and $^{33}\text{P}$ counting channels. The d.p.m. of each isotope were calculated by reference to previously prepared quench curves, after correction for background radioactivity and for the cross-over of the more energetic $^{32}\text{P}$ radioactivity to the $^{33}\text{P}$ counting channel.

**Deconvolution analysis**

The mathematical technique of deconvolution was used to calculate phosphate absorption after simultaneous oral and intravenous doses of the phosphorus isotopes. The method used is based on that of Hart & Spencer (1967) for the measurement of intestinal absorption of calcium and strontium, and a brief statement of the principle of the technique follows.

$F(t)$ and $G(t)$ are the plasma radioactivity curves after intravenous administration of $^{33}\text{P}$ and oral administration of $^{32}\text{P}$ respectively, given at time zero. $G(t)$ may be regarded as a superimposition of plasma radioactivity curves of all the individual differential initial entries $B(r)dr$ from the gastrointestinal tract to the blood stream, assuming that $B(r)$ is the initial rate of entry of $^{32}\text{P}$ and $dr$ is the differential time interval.
Radioisotope measurement of phosphate absorption

during which entry takes place. [Introducing a tracer by the oral route is equivalent to labelling the vascular pool with a continuous intravenous infusion rather than a single intravenous injection, and \( B(t) \) is the variable rate of intravenous infusion which would give rise to the same blood curve as is observed after an oral dose.] The above terms are linked by eqn. (1).

\[
G(t) = \int_0^t B(\tau)F(t - \tau)\,d\tau \tag{1}
\]

\( G(t) \) and \( F(t) \) are determined experimentally, and this expression can be reduced to a set of simultaneous equations, which when solved give the percentage of the oral dose of \( ^{32}\text{P} \) absorbed per 15 min interval. These values can be summed to ascertain the total percentage of oral dose absorbed.

Use of this method enabled three estimates of phosphate absorption to be obtained for each patient. The maximal rate of phosphate absorption was taken as the maximal percentage of oral dose of \( ^{32}\text{P} \) absorbed per 15 min interval (Reeve et al., 1974). Values for cumulative percentage absorption at 3 and 7½ h were also calculated.

**Phosphate absorption estimate based on \( ^{32}\text{P} \) alone**

Estimates of phosphate absorption were obtained from single values of the plasma activity (percentage of dose/l of plasma) of \( ^{32}\text{P} \) at 30, 60, 90 and 120 min. These values were corrected for body weight to be expressed as the fraction of the administered dose of \( ^{32}\text{P} \) present in the extracellular fluid and were designated \( P30, P60, P90 \) and \( P120 \) respectively. The fractional hourly rate of intestinal phosphate absorption (\( aP \)) was calculated as previously described (Marshall, 1976; Farrington et al., 1979a). The area under the corrected plasma radioactivity curve of \( ^{32}\text{P} \) up to 2 h was also calculated and expressed as \( PA \).

**Statistical analyses**

Tests for normality of distribution of results (Rankit method) in the various groups considered, and in all the group considered together, revealed that most distributions did not conform to normality. Non-parametric statistics were thus applied throughout. The statistical differences between the results of the various groups were assessed by means of the Wilcoxon rank sum test. The estimates of phosphate absorption obtained by deconvolution analysis and those obtained from plasma radioactivity levels of the orally administered isotope alone (\( P30, P60, P90, P120 \) and \( aP, PA \)) were compared by calculation of the Spearman Rank correlation coefficient (\( r_s \)).

**Results**

The plasma \( ^{33}\text{P} \) and \( ^{32}\text{P} \) radioactivity curves are shown in Figs. 1 and 2 respectively. Although the plasma \( ^{33}\text{P} \) radioactivity curves were of a similar configuration in all groups, the curve in the chronic renal failure group differed significantly (\( P < 0.01 \) at all points) from control curves. The curve in transplant patients did not differ significantly from control curves at any point. The plasma \( ^{32}\text{P} \) radioactivity curve (Fig. 2) showed a peak at 1 h in both transplant patients and controls, though in transplant patients the points were significantly lower than in controls at 1 h (\( P < 0.05 \)) and at 1⅓, 2 and 3 h (\( P < 0.01 \)). The peak in the plasma \( ^{32}\text{P} \) radioactivity curve in chronic renal failure patients appeared later at 2 h, and this curve only differed from the control curve at 1 h (\( P < 0.05 \)).

Fig. 3 shows the intestinal absorption of phosphate as estimated by deconvolution analysis and expressed as percentage absorption of \( ^{32}\text{P} \) oral dose per 15 min interval. In all three groups the pattern of absorption was similar; percentage phosphate absorption was greatest at 30 min, and after 3 h continued at a low level which was very similar in all groups. The greatest difference between the curves occurred in the first 2 h, during which phosphate malabsorption was demonstrated in both chronic renal failure and transplant groups.

Individual values for the maximal rate of phosphate absorption, and for cumulative phosphate absorption at 3 and 7½ h obtained by deconvolution analysis, are shown in Fig. 4. Control values for cumulative phosphate absorption at 3 and 7½ h fell into a tight range, but for maximal phosphate absorption the range was much wider. In both the chronic renal failure group and the transplant group there was a wide scatter of values for all three parameters of phosphate absorption, ranging from normal to values indicative of gross phosphate malabsorption. There were statistically significant differences between both chronic renal failure and transplant patients versus controls with respect to all three parameters of phosphate absorption obtained from deconvolution analysis (\( P < 0.01 \) in all cases). The maximal rate of phosphate absorption was highly correlated with cumulative phosphate absorption at 3 h (\( r_s = 0.91, P < 0.001 \)) and 7½ h (\( r_s = 0.84, P < 0.001 \)). Cumulative absorption at 3 h was highly correlated with that at 7½ h (\( r_s = 0.92, P < 0.001 \)).
FIG. 1. Mean plasma $^{33}$P radioactivity curve. Vertical bars indicate SEM of points. ●, Controls; ▲, chronic renal failure patients; ■, post-transplant patients.

FIG. 2. Mean plasma $^{32}$P radioactivity curve. Vertical bars express SEM of points. ●, Controls; ▲, chronic renal failure patients; ■, post-transplant patients.
Radioisotope measurement of phosphate absorption

Fig. 3. Mean percentage administered dose of $^{32}$P absorbed per 15 min intervals obtained by deconvolution analysis. Vertical bars express SEM of points. ●, Controls; ▲, chronic renal failure patients; ■, post-transplant patients.

Fig. 4. Individual values of phosphate absorption obtained by deconvolution analysis. A. Controls; B. chronic renal failure patients; C. post-transplant patients.
FIG. 5. Mean corrected plasma levels of $^{32}$P. Vertical bars indicate SEM of points. ●. Controls; ▲. chronic renal failure patients; ■. post-transplant patients. Significance of results: ⚫. $P < 0.01$; ●. $P < 0.05$. ECF, Extracellular fluid.

FIG. 6. Mean fractional hourly rate of absorption $(aP)$ and mean area under corrected plasma radioactivity curve $(PA)$ in the three groups (A. controls; B. chronic renal failure patients; C. post-transplant patients). Vertical bars indicate SEM of points. N.S., Not significant.
Estimates of phosphate absorption, based on the corrected plasma radioactivity of $^{32}$P only, at various times are shown in Fig. 5. The area under the corrected plasma $^{32}$P radioactivity curve up to 2 h and the fractional hourly rate of absorption of $^{32}$P are shown in Fig. 6. There were significant differences ($P < 0.01$) between the transplant group and controls with respect to all the corrected plasma levels from 60 min to 7½ h (Fig. 5). However, the chronic renal failure patients could only be distinguished from the control group with respect to the corrected plasma radioactivity level at 60 min ($P < 0.05$). The transplant group could also be distinguished from controls with respect to the area under the corrected plasma radioactivity curve ($P < 0.01$). However, there were no significant differences with respect to this parameter between chronic renal failure and control groups (Fig. 6). Both the transplant group ($P < 0.01$) and the chronic renal failure group ($P < 0.05$) could be distinguished from controls with respect to the fractional hourly rate of absorption (Fig. 6).

The estimates of phosphate absorption based on the analysis of one or more plasma $^{32}$P radioactivity levels were then compared with those obtained by the deconvolution method by means of Spearman rank correlation coefficient. The results of this comparison can be seen in Table 1. There were highly significant correlations ($P < 0.001$ in all cases) between $P_{30}$, $P_{60}$, $P_{90}$, $P_{120}$, $aP$ and $PA$ and the maximal rate of phosphate absorption and both the cumulative percentage phosphate absorption at 3 and 7½ h. The highest degrees of correlation with the maximal rate of phosphate absorption were obtained for $aP$, $P_{30}$, $P_{60}$, $PA$. The highest degree of correlation with cumulative phosphate absorption at both 3 and 7½ h were obtained for $aP$, $P_{60}$, $PA$.

### Discussion

The plasma $^{33}$P radioactivity curve is of a similar shape in all three groups, although in the chronic renal failure group the plasma radioactivities are consistently higher than in the control and transplant groups (Fig. 1). If complete mixing in the blood compartment has occurred before the first sample at 15 min, then the shape of the curve will be largely determined by the rate of exit of $^{33}$P from the vascular compartment and its rate of return. The constantly higher plasma radioactivities in the chronic renal failure group could be caused by a combination of factors. A diminished rate of exit of phosphate from the vascular compartment due to diminished renal excretion and to diminished fluxes to the tissue compartment could contribute to a small available volume of distribution for phosphate in chronic renal failure patients. There could also be a 'dilutional' effect on the concentration of $^{33}$P within the plasma as a consequence of the raised plasma phosphate levels in chronic renal failure, causing an apparent diminished fractional rate of exit of the isotope from the vascular compartment.

Similar factors are important in determining the shape of the plasma $^{33}$P radioactivity curves, but here an important additional factor is the rate of intestinal absorption of phosphate. The shape of the plasma $^{32}$P radioactivity curves is similar in transplant patients and controls and it may be that the major difference between the curves, i.e. the height of the peak, reflects differences in the intestinal absorption of phosphate. However, the shape of the plasma $^{32}$P radioactivity curve in chronic renal failure patients is markedly different from the control curve, suggesting there are major differences in phosphate kinetics between the two groups other than differences in phosphate absorption. Such factors require con-
sideration before inferences about phosphate absorption can be drawn from data based on plasma $^{32}$P radioactivity curves alone. Deconvolution analysis takes these factors into account.

We have described the adaptation of a deconvolution method previously reported for the measurement of intestinal calcium and strontium absorption. The basis of the deconvolution method is that the major differences between the fate of orally versus intravenously administered phosphate can be accounted for by intestinal absorption. It is known, however, that intraluminal phosphate may be incorporated into mucosal cell phospholipids (Thompson & Deluca, 1964; Wasserman & Taylor, 1973), and the further assumption that only a small proportion of orally administered phosphate is trapped in the gut mucosa by such mechanisms is implied.

A further point of inaccuracy is that the cumulative percentage absorption of phosphate is calculated by assuming absorption to have been completed at 7½ h. However, it is apparent (Fig. 3) that even at this late stage absorption is continuing at a slow rate in all three groups, and this would tend to cause an under-estimation of total phosphate absorption. However, the errors due to this are unlikely to be large. Although it is not possible from our data to assess the accuracy of this method as applied to phosphate absorption, calcium absorption as estimated by this method has been shown to correlate well with absorption measured by faecal excretion of radiochloride (Hart & Spencer, 1967).

From Fig. 3 it is also apparent that $^{32}$P absorption occurs in two distinct phases. First, there is a rapid phase of absorption during which, in controls, greater than 50% of the orally administered dose is absorbed during the first 2 h. The second phase of absorption is one in which a low fairly constant absorption rate continues over a prolonged period of time. Phosphorus absorption has been considered to occur predominantly by active transport processes (Harrison & Harrison, 1961; Helbock, Forte & Saltman, 1966; Wasserman & Taylor, 1973), although simple diffusion processes have been thought to be more important by others (McHardy & Parsons, 1956; Hurwitz & Bar, 1972). However, it has been suggested that phosphate absorption, like calcium absorption, involves both processes (Wilkinson, 1976). It is possible that the data presented here are also consistent with this latter view, the initial rapid absorption phase corresponding to an active process which is impaired in chronic renal failure and transplant patients, and the more prolonged slower phase of absorption representing the passive process which is similar in all three groups. The influences of vitamin D metabolites on each of these phases of absorption is relatively unknown, though we have previously demonstrated that 1,25-dihydroxycholecalciferol is effective in reversing phosphate malabsorption in some patients with primary biliary cirrhosis (Farrington et al., 1979a), and that 1α-hydroxycholecalciferol was ineffective in chronic renal failure patients (Varghese, Farrington & Moorhead, 1979). In both these studies phosphate absorption was assessed by the fractional hourly rate of phosphate absorption based on the administration of oral $^{32}$P (Marshall, 1976), so that any changes observed with vitamin D therapy would reflect changes in the initial rapid phase of absorption.

Phosphate malabsorption has been previously described in chronic renal failure patients (Stanbury, 1976; Coburn, Brickman, Hartenbower & Norman, 1977; Farrington et al., 1979b) and also in transplant patients (Farrington et al., 1979b). The causes of phosphate malabsorption in the latter are unknown, but it may be that the interaction of various drugs with intestinal phosphate-transport mechanisms is important, and both steroids and cyclophosphamide have been shown to reduce phosphate absorption (Cannigia & Gennari, 1971). Other factors such as the systemic hyperchloraemic acidosis seen in some transplant patients (Moorhead, Ahmed, Varghese, Wills, Baillod, Tatler & Fairney, 1974) could also contribute.

The maximal rate of phosphate absorption by deconvolution analysis has been shown to be highly correlated with both the cumulative percentage absorption of phosphate at 3 h ($r_c = 0.91, P < 0.001$) and that at 7½ h ($r_c = 0.84, P < 0.001$), implying that the initial rate of phosphate absorption is closely related to the total fraction of phosphate absorbed. In addition all three of these parameters of phosphate absorption derived from deconvolution analysis were able to distinguish between the three study groups with the same degree of probability ($P < 0.01$). Hence sampling up to 3 h would seem to provide an adequate assessment of phosphate absorption by this method, allowing for calculation of the maximal range of absorption, and the cumulative percentage absorption at 3 h. However, to study the differential effects of various compounds on the different phases of absorption, the longer sampling time would be necessary.

Of the estimates of phosphate absorption derived from analysis of the plasma $^{32}$P radioactivity alone, only $\alpha P$ and $P_60$ can discriminate satisfactorily between chronic renal failure
Radioisotope measurement of phosphate absorption

References


