STUDIES IN MINERAL METABOLISM WITH THE AID OF ARTIFICIAL RADIOACTIVE ISOTOPES*

I. ABSORPTION, DISTRIBUTION, AND EXCRETION OF PHOSPHORUS

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Prior to 1935, studies on the absorption of phosphorus were carried out either by the use of intestinal fistulas, or by the addition of an inactive unabsorbed marker to the food, such as iron oxide in the method of Bergeim (1). Recently the excretion of calcium and phosphorus into the large intestine of the rabbit has been studied by Cowell (2) by means of the differential analysis of fecal pellets and changes in the concentrations of these elements from cecum to anus. With the discovery of induced radioactivity (3), and the preparation of the radioactive phosphorus isotope of atomic weight 32 (4), it has become possible to investigate the rates of absorption, distribution, and excretion of phosphorus, and the percentages involved. One can now distinguish the administered phosphorus from that present in the animal under investigation (5, 6).

Hevesy, in 1923, demonstrated the use of radioactive isotopes as indicators in biological investigations (7), and Chiewitz and Hevesy were the first to use the P³² isotope in studies of phosphorus metabolism (6, 8). Since then, with the invention and development of the “cyclotron” by Lawrence and collaborators (9, 10), and the resultant availability of “radiophosphorus,” work has been published on certain phases of phosphorus storage and elimination (6, 8, 11–13), on the rate of phospholipid synthesis (14–17), and upon the locale of this synthesis (17).

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In the majority of these studies measurements were not made until 1 or more days had elapsed following the phosphate administration, at which time the phosphorus administered had reached a relatively steady state with respect to that formerly present in the animal. In only one case (13) had the rates of absorption and excretion, which occur mainly in the first 24 hours, been investigated. Accordingly, most of the present work has been confined to the changes occurring in the first 48 hours following the administration, orally or by intraperitoneal injection, of a single dose of neutral sodium phosphate, particular attention being paid to the rate and amount of excretion and the initial movements and storage of the phosphorus.

Methods

The animals used were rats weighing from 220 to 300 gm. and falling into two age groups of 70 to 100 and 100 to 130 days, respectively, at the time of use. Each animal was fasted 12 hours before being given the dose of phosphate. 1 or 2 hours after administration of the phosphate the rat was given access to food.

The radiophosphorus was prepared from ordinary red phosphorus by bombardment with deuterons in the cyclotron of the Radiation Laboratory of the University of California. After the bombardment the sample was digested with dilute HCl to remove traces of aluminum, filtered, and then oxidized to \( \text{H}_3\text{PO}_4 \) with \( 8 \text{ N HNO}_3 \). This was evaporated on a steam bath and the excess \( \text{HNO}_3 \) decomposed with concentrated HCl. The solution was again evaporated, diluted, and neutralized with NaOH (heavy metals, if present, were precipitated with \( \text{H}_2\text{S} \)) to a pH of about 8; i.e., acid to phenolphthalein and alkaline to litmus. The resultant phosphate solution was made up to such a concentration that 1 ml. contained around 1000 to 2000 times the minimum quantity of \( ^{32}\text{P} \) which could be detected on the electroscope. This volume constituted a single dose. The amount of phosphate given per dose was therefore dependent upon the relative radioactivity of the sample used and the sensitivity of the measuring device. In these experiments the dose varied from 4.5 to 13 mg. of phosphorus.

After administration of the phosphate, each animal was placed in a separate cage over a urine-feces separator (18). At the end
of the desired period, measured from the time of administration, the animal was anesthetized with chloroform and bled by heart puncture, following which the tissues to be examined were dissected out. These tissues and the urine, feces, and blood samples were then ashed with Mg(NO$_3$)$_2$ at 500° (19). The ash was dissolved in HCl or HNO$_3$, and the phosphate in an aliquot containing about 5 mg. of P was precipitated, first as ammonium phosphomolybdate, then as magnesium ammonium phosphate (19). The MgNH$_4$PO$_4$·6H$_2$O was filtered through a Whatman No. 50 filter paper, dried at 120-140°, and transferred quantitatively to a 3 × 4 cm. copper tray. The filter paper was ashed and added to this and the whole was then spread uniformly over the surface of the tray. This precaution, and the use of 5 mg. aliquots of phosphorus in each case, kept the error due to self-absorption of β-rays at a low, and nearly constant level, for the thickness of the measured powder was only a fraction of a mm. Radioactivity was measured by means of a Lauritsen electroscope in which an aluminum foil window had been inserted. Aliquots of the solution of phosphate administered, equal in phosphorus content to the unknown samples, were analyzed in the same manner for P$^{32}$, and served as the standard of comparison for the unknown samples.

The radioactivity was measured in arbitrary units and, after correction for decay, is expressed in terms of the per cent of the radioactivity of the administered phosphate. Total phosphorus, when it was measured, was determined on the ash by the colorimetric method of Fiske and Subbarow (20), as modified by King (21).

The experiments carried out here were divided into four series. Series I consisted of sixteen rats of the older group which were given the phosphate by stomach tube. Series II consisted of eight rats of the same age group which received the phosphate by intraperitoneal injection. In Series III four rats of the younger group were given the phosphate by stomach tube. Series IV was a study of excretion in both age groups, the phosphate being administered by stomach tube, by intraperitoneal injection, or mixed with food. Urine and feces were collected at short intervals and analyzed separately for about 5 days thereafter.

Rats in Series I received 6 to 13 mg. of P, the majority (ten out of sixteen) receiving 12 mg. of P. The animals in Series II and
III received 4.5 mg. of P (a stronger sample of P\textsuperscript{32} was used here), while those in the experiments on excretion in Series IV received from 4.5 to 28 mg. of P.

**Results**

*Absorption*—In Fig. 1 is plotted the percentage of unabsorbed phosphorus found in the gastrointestinal tracts of the rats of Series I and III. In Series I the rats were given access to food 1 hour after administration of the phosphate, which may or may not be the reason for the delayed absorption as compared to the rats of Series III where 2 hours elapsed.

The unabsorbed phosphorus was calculated by subtracting the amounts found in each section of the gut of animals which had been injected intraperitoneally with the same amount of phosphate and killed following the same time interval. In other words, the values in Series II (varying from 0.5 to 2 per cent) were subtracted from the corresponding values in Series I and
III in order to correct the latter for radiophosphorus secreted or excreted into the intestines from previously absorbed material.

The major portion of the absorption occurs within the first 2 hours and the stomach is usually empty of radiophosphorus within 4 hours. In no case was any unabsorbed radiophosphorus found in the stomach at or after 8 hours. The small intestine naturally is cleared more slowly of phosphorus, but in only one case (at 9 hours) was any unabsorbed radiophosphorus found in the small intestine after 8 hours.

Although not yet appearing in the feces, radiophosphorus is found in the large intestine and cecum within 2 hours after administration. These figures also represent only unabsorbed material, having been corrected for the amount excreted from the blood into the large intestine as estimated from Series II. Within 4 hours after ingestion, the major portion of the administered radiophosphorus not retained by the tissues has found its way into the urine and contents of the large intestine.

**Distribution**—The major portion of the absorbed phosphorus is taken up by the muscle and bone, as might be expected from their relative mass and high phosphorus content, respectively. The amounts involved could not be estimated very accurately owing to the errors involved in sampling and measuring a small aliquot. Muscle, on the basis of the analysis of one gastrocnemius in each animal, and assuming a muscle content of 43 per cent of the body weight (21), took up about 15 per cent in 4 hours. Subsequently the amount diminished slowly to about 10 to 12 per cent in 150 hours. From femur analyses, assuming this to represent the 6.1 per cent of bone in the rat (21), bone attains a value of 20 to 25 per cent in 2 to 4 hours, dropping to around 15 per cent after 150 hours. After 4 to 8 hours the carcass, by which is meant the residue after removal of the viscera and their contents but including both muscle and bone, contained a maximum of about 60 to 65 per cent of the absorbed phosphorus, following which there is a drop to 50 or 55 per cent in 48 hours.

Among the viscera, liver takes up the largest fraction of the absorbed phosphorus, followed by the stomach and small intestine (considered together). The accumulation and withdrawal from these organs are shown in Fig. 2 in terms of per cent of phosphorus injected intraperitoneally (curve IP) and per cent of phos-
Phosphorus given by stomach tube (curve ST). When the latter values are corrected for the amounts not absorbed from the intestinal tract, they approach the values found in the injection series. Recent work has shown that the small intestine accounts for most of the total.

Over half of the phosphorus accumulating in the liver in the first 6 hours is in the form of newly synthesized phospholipid, as shown by Perlman, Ruben, and Chaikoff (17). Only a very small portion of this can be due to the blood in the liver, as the blood at no time contains more than 4 per cent of the total radiophosphorus in its entire volume.

Stomach and small intestine show differently shaped curves for the two types of administration. The sharp rise and abrupt fall obtained when the phosphate is given by stomach tube may be explained by the superimposition of absorption upon accumulation. This may also explain the very high values found during the period of 2 to 4 hours when the figures are expressed in terms of the amount of phosphorus absorbed as compared to the data.

![Graph showing retention of radiophosphorus in liver and in stomach and small intestine](http://www.jbc.org)
from the injection method. In these tissues, as in liver, a large part of the radiophosphorus found is in the form of phospholipid (17).

The per cent of absorbed radiophosphorus found in the blood varied inconsistently from 2.6 to 4.0 per cent after 2 hours to around 1 per cent after 48 hours had elapsed.

In Fig. 3 are plotted the radiophosphorus contents of kidneys, lungs, heart, and brain. Owing to the small amounts no high degree of accuracy is claimed for these measurements; they are included to show the general trends and approximate affinities of these organs for absorbed phosphate. Of interest are the retentions of phosphorus by kidney and brain. The former shows a rapid rise and a fairly rapid fall in radiophosphorus content, but the fall is not so rapid as is that of the urinary excretion. Brain shows a lag in accumulation and a very slow turnover of phosphate, a fact noted by Artom et al. (14).

More important than the phosphorus retention of the whole
of a particular tissue or organ is the "specific affinity;" i.e., the retention (in per cent of absorbed radiophosphorus) per gm. of the fresh tissue. Only when the foregoing figures have been reduced to this basis, can the retentions of the different tissues be rightly compared. These are given in Figs. 3 and 4.

As noted in the cases of kidneys, lungs, heart, and brain, the figures given for bone, muscle, and carcass are significant only as an indication of the order of magnitude of the phosphorus retention of these tissues.

Skin, including hair and adherent subcutaneous tissue, showed a slow rise from a content of 0.05 to 0.09 per cent of absorbed radiophosphorus per gm. in 40 hours.

Bone shows the largest specific affinity of any tissue studied, although, when reduced to unit phosphorus content, it becomes the lowest. Liver, stomach and small intestine, heart, kidneys, lungs, carcass, blood, muscle, and brain show decreasing specific affinities in this order. The typical sharp rise and gradual fall in radiophosphorus content are observed in all cases with the exception of brain.

Excretion—In Fig. 5 are plotted the radiophosphorus contents of the urine and feces for the rats in Series I to IV in per cent of the total administered. Curves 1, 2, and 3 represent the per cent excreted in the urine under the various conditions up to the times noted. Curves 4 to 8 show the corresponding fecal excretions.

Curve 1 is drawn to cover two series of points, representing 4.5 and 9 mg. of P, respectively, injected intraperitoneally. The 9 mg. points are the averages of two animals in Series IV. The 4.5 mg. points are the averages of one animal in Series IV and the eight individual rats in Series II.

Curve 2 represents the urinary excretion, in per cent of total radiophosphorus administered, for the same amounts given by stomach tube. The 4.5 to 6 mg. of P points represent the averages of experiments on one rat of Series IV and four rats of Series I (6 mg. of P) run continuously in the same manner as those in Series IV. The 9 mg. points are the averages found for two rats in Series IV. One curve fits both series of points and this curve, if corrected for the amount unabsorbed (average = 30
Fig. 4. Retention of radiophosphorus in various tissues and in blood per gm. of fresh weight. A, administered by intraperitoneal injection; • Series II, □ Series I and III (stomach tube) in terms of per cent of absorbed radiophosphorus. B, administered by stomach tube; • Series I (6 to 13 mg. of P), ○ Series III (4.5 mg. of P).
per cent; factor = 1.4), will approach the injection curve quite closely.

Curve 3 represents urinary excretion in two rats of Series IV following the administration of 28 mg. of P in 3 gm. of normal rat food. It rises a bit more slowly owing to slower absorption, but approaches Curve 2 as time increases.

Curve 4 represents the per cent of administered phosphate found in the large intestine, cecum, and feces together. Each point is the average for two rats, one of Series I and one of Series III. Each individual value is within one unit of the average value given.

Curve 5 is drawn from the average of the values obtained from two rats in Series IV, given 4.5 and 9 mg. of P, respectively, and the averaged values of four rats in Series I (6 mg. dose) measured continuously. This curve approaches the plateau of Curve 4 more slowly, as it represents feces only.

Curve 6 is the fecal excretion of the two rats given the radiophosphorus in food and, as expected, shows a still slower rise to the maximum.
Curve 7 represents the sum of the amounts found in large intestine, cecum, and feces in the rats of Series II, injected intraperitoneally with 4.5 mg. of P. Each point represents a separate rat.

Curve 8 represents the averaged values of three rats of Series IV, two of which were given 9 mg. of P intraperitoneally, the third receiving 4.5 mg. of P in the same manner.

The large fecal excretion of ingested phosphorus, shown in Curves 4, 5, and 6, represents mainly unabsorbed phosphorus, for the 70 per cent which was absorbed would follow the same course as the 100 per cent injected and would give the same small amount of radiophosphorus in the feces shown in Curves 7 and 8. However, it cannot be said that excretion or secretion of absorbed or injected phosphate into the intestine and its subsequent excretion via the feces is negligible, a hypothesis that may be suggested by the latter two curves. Once absorbed into the blood, the radiophosphorus administered marks not only the 4.5 or 9 or 12 mg. of phosphorus associated with it at the start, but a larger amount limited only by the total phosphorus in the body. In other words, the active phosphorus becomes "diluted" with inactive phosphorus in the blood and tissues, and we can then no longer say that 2 per cent of the administered active phosphorus represents 2 per cent of 4.5 or 9 or 12 mg. of phosphorus. Until both this dilution and that of unabsorbed P$^{32}$ due to digestive juices are measured, no estimate of the relative amount of phosphorus in the feces due to lack of absorption and that due to excretion from the organism may be made.

Although we cannot yet place an absolute value on the fecal excretion of absorbed phosphorus, we can determine the ratio of urinary to fecal excretion of this material directly from the curves. Assuming that both urine and intestinal secretions are removed from the blood at about the same rate and time, the ratio of P$^{32}$/P$^{31}$ will be the same in each. Therefore the ratio P$^{31}_f$/P$^{31}_u$ for absorbed phosphorus (where P$^{31}_f$ = total P added to the contents of the large intestine during the interval measured, and P$^{31}_u$ that added to the urine) will equal the ratio P$^{32}_f$/P$^{32}_u$, which is shown by the values of Curves 8 or 7 over those of Curve 1 (Fig. 5). At 48 hours this ratio is about 1:10. That is to say, only about 1/11 of the absorbed phosphorus excreted by the body
in this time is eliminated via the gut and feces, while 10/11 is excreted via the urine. This is in fair agreement with the ratio found by Chiewitz and Hevesy (8) in humans. Furthermore, if the ratio $P_f^1/P_u^1$ is known, the determination of $P_u^1$ allows $P_f^1$ to be calculated and a definite value thus to be placed on the truly excreted fecal phosphorus. In like manner the absolute phosphorus uptake or turnover of an organ or tissue in any given length of time may be calculated.

Worthy of note are the rapid rises in Curves 1, 2, 3, 4, and 7, showing that the major portion of the administered phosphorus not retained by the tissues finds its way into the large intestine or urine within the first 4 to 8 hours, subsequent elimination being very slow. These facts, coupled with those shown by the retention curves, support our starting hypothesis that the first 24 hours following administration are the most important ones for studying the movements of a single dose of phosphorus.

**SUMMARY**

1. Study of phosphorus metabolism in rats with the aid of radiophosphorus has shown that the major disposition of injected or ingested phosphate occurs within the first 8 hours after administration.

2. Absorption of phosphorus, administered as dissolved Na$_2$HPO$_4$, is most rapid in the first 2 hours and is usually at an end 8 hours after ingestion. The absorption is never complete, about 30 to 40 per cent of the ingested phosphorus remaining unabsorbed.

3. Of the injected or absorbed phosphorus about 20 to 30 per cent is excreted in the urine within 8 hours, while about 3 per cent is excreted via the large intestines. The subsequent elimination of this phosphorus over a 5 day period in both urine and feces goes on at a steadily diminishing rate that amounts to about 2 to 1 per cent per day.

4. The tissues which were examined retain the absorbed or injected phosphorus in the following decreasing order: bone, muscle, liver, stomach plus small intestine, blood, kidneys, heart, lungs, and brain. All except brain show a rapid uptake in the first 10 hours, followed by a prolonged, steadily diminishing loss. Brain apparently has a very slow turnover of phosphorus.
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5. Per unit of fresh weight of tissue the retentions—called the "specific affinities"—assume the following order: bone, liver, stomach plus small intestine, heart, kidneys, lungs, blood, muscle, skin, and brain.

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