

MIXING RATE OF PHOSPHATE BETWEEN PLASMA AND INTERSTITIAL BODY FLUID OF COWS*

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Isotopes as tracers offer the possibility of studying the kinetics of phosphate metabolism in the normal intact animal. Our aim in such a study on dairy cows is to describe the cow's phosphorus exchange by a system of quantitative terms, especially capacities and rates of exchange. According to the varying mobility, the total amount of phosphorus in the animal may be partitioned among a series of pools. Each of these pools may be characterized by its exchange rates with other pools and its capacity. Biochemically, it is of interest to establish the correlation between the chemical nature of phosphorus compounds in the body, the metabolic processes involved, and the exchange rates. Physiologically, it is important to study the distribution of the various pools among the organs and possibly to correlate physiological function with pool capacity and exchange rate.

This paper deals mainly with the analysis of the decrease of radioactivity with time in plasma from cows for the first 20 minutes after intravenous injection with radioactive phosphate. The data are interpreted as the result of mixing of plasma phosphate with the phosphate of the interstitial body fluid.

In order to avoid confusion between postulates and conclusions, we shall number, instead of name, the phosphate pools and the exchange processes dealt with in this paper. The tracer, P^{32} , is injected as sodium phosphate into the first pool whose capacity is the phosphate content of the plasma. The second pool is a postulated phosphate reservoir which is in most rapid exchange with the first pool. The capacity of pool 2 is derived as an empirical constant from the time relation of tracer activity in the first pool. Correspondingly we distinguish 3 processes of different rapidity: *Process 1* is the mixing of the tracer within the first pool (plasma). This process presumably is practically completed at the starting time of our calculations (5 minutes after injection).¹ *Process 2* is the mixing of phosphate between the first and second pool. This process takes place mainly between 5 and 20 minutes after injection. *Process 3* is characterized by the loss of activity from the first (or second) pool after the mixing between these

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¹ Dobson, E., and Jones, H. B., personal communication.

two is completed. Process 3, however, presumably takes place also in the period 5 to 20 minutes after injection, either parallel to process 2 or in series with it.

Method

Cows.—The basis for our calculations is two trials with two lactating Jersey cows which had served for earlier trials on secretion of injected P^{32} in milk.² One trial (VII) was carried out with a 3 year old cow weighing 380 kilos which had calved 7 months before the trial and was producing about 8 liters of milk per day. The cow used for the other trial (VIII) was $7\frac{1}{2}$ years old, weighed 400 kilos, had calved 7 months before the trial started, and still produced nearly 10 liters of milk per day.

Procedure.—One dose of 41 millicuries of radioactive phosphate in one trial, and 38 millicuries in the other, contained in isotonic saline solution, was injected into the left jugular vein of each of the two cows, through a plastic tube, known as electrician's spaghetti.³ The plasma volume of the cows was measured by injecting a 1 per cent aqueous dispersion of Evan's blue into the vein and measuring the concentration of the dye in the plasma at different times from injection with the aid of a spectrophotometer, extrapolating the results to time of injection, assuming the decrease in plasma to follow a first order reaction.⁴ The P in plasma ash was measured by the method of Fiske and SubbaRow.⁵ The concentration of the tracer was measured in the plasma directly and also in the trichloroacetic acid filtrate of the plasma. The latter results (P^{32} in acid-soluble phosphorus fraction of plasma) appeared less uniform and not significantly different from those with regard to the P^{32} in plasma measured directly. This study, dealing with the first 30 minutes, therefore, was based on P^{32} in the plasma, rather than in the trichloroacetic acid filtrate. During this period, presumably we had to do almost exclusively with acid-soluble phosphate since the turnover to other phosphorus compounds, especially the phospholipids, is considerably slower.^{6,7}

RESULTS

Plasma Volume.—The plasma volume of the 3 year old cow used in trial VII was 17.3 liters or 4.6 liters per 100 kilos of body weight. The $7\frac{1}{2}$ year old cow used in trial VIII had a plasma volume of 26.2 liters or 6.5 liters per 100 kilos of body weight.

P^{32} in Plasma.—Fig. 1 shows the P^{32} concentration per liter blood plasma in per cent of the total injected dose, plotted against time after injection.

The curves seem to indicate that the P^{32} , injected into the jugular vein, is

² Kleiber, M., Smith, A. H., and Ralston, N. P., *Proc. Soc. Exp. Biol. and Med.*, 1948, **69**, 354.

³ Ralston, N. P., Kleiber, M., and Smith, A. H., *J. Dairy Sc.*, 1949, **32**, 889.

⁴ We are indebted to E. Dobson for some of these measurements and calculations.

⁵ Fiske, C. H., and SubbaRow, Y., *J. Biol. Chem.*, 1925, **66**, 375.

⁶ Fishler, M. C., Entenman, C., Montgomery, M. L., and Chaikoff, I. L., *J. Biol. Chem.*, 1943, **150**, 47.

⁷ Hevesy, G., *Radioactive Indicators*, New York, Interscience Publishers, Inc., 1948, 305.

uniformly distributed about 3 minutes after injection. At this time a considerable amount of P^{32} appears to have left the plasma already. Thus in trial VII only 28 per cent, in trial VIII only 41 per cent of the injected dose is accounted for by the radioactivity in the plasma 3 minutes after injection.

Mixing Rate and Pool Capacity.—Zilversmit, Entenman, and Fishler⁸ have developed a general differential equation for calculating turnover rates from the time relation of specific activities. The relative turnover rate of a given component (or the reciprocal of its turnover time) is the rate of increase in specific activity of the component divided by the difference in specific activity of component and its precursor. For estimating mixing rates of phosphate between plasma and second pool, we assume that during the first hour after injection

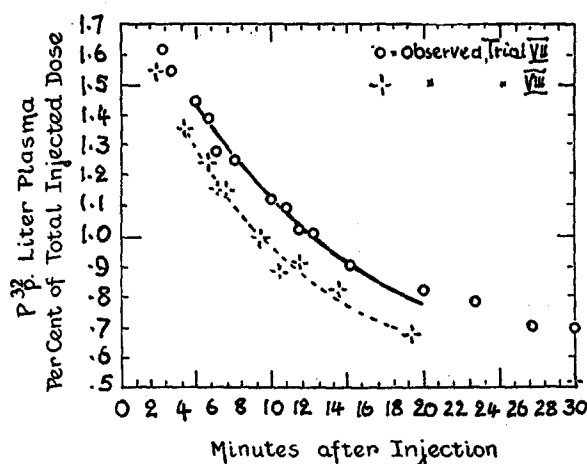


FIG. 1. P^{32} in blood plasma of cows.

tion of the tracer the phosphate content of the plasma remains constant. In this case the specific activity of the plasma is directly proportional to the P^{32} content per ml. plasma, and the calculations may be carried out with millicuries per ml., or with P^{32} per ml. plasma in per cent of the injected dose. In this case also a liter of plasma represents a given amount of phosphate, therefore the capacity of the plasma phosphate pool may be expressed in liters of plasma.

Process 3.—Various processes contribute to the decrease of activity in the blood plasma. Fig. 2 in which the logarithm of the plasma activity is plotted against time, shows, however, that between 20 and 60 minutes after injection the log of plasma activity may be regarded as a linear function of time, that therefore all the processes decreasing the plasma activity in this period may be summarized as a first order reaction with regard to plasma activity. For trial

⁸ Zilversmit, D. B., Entenman, C., and Fishler, M. C., *J. Gen. Physiol.*, 1942, **26**, 325.

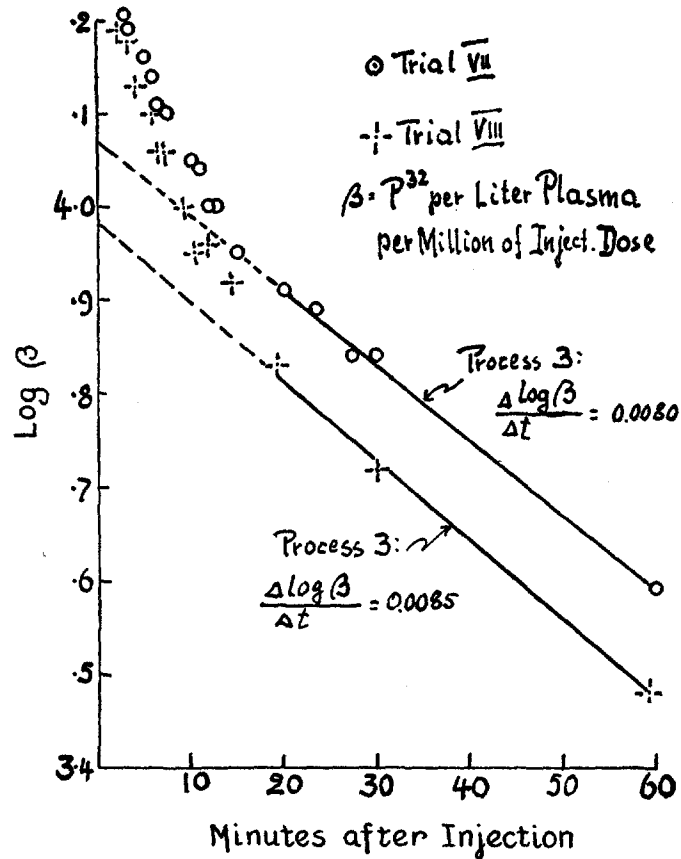


FIG. 2. Log of P^{32} in plasma of cows as a function of time.

VII the regression coefficient for this part of the curve, $\frac{\Delta \log \beta}{\Delta t}$ amounts to 0.008, thus $\frac{\Delta \ln \beta}{\Delta t} = 0.019$ and consequently

$$\frac{d\beta}{dt} = 0.019\beta,$$

where β is the activity in 1 liter plasma per million of the total injected dose and 0.019 the relative turnover rate. For trial VIII, similarly the relative turnover rate amounts to 0.020. Therefore, the turnover time (the reciprocal of the relative turnover rate) of process 3 was 53 minutes for trial VII and 50 minutes for trial VIII.

Process 2.—One may now assume that process 3, just described, takes place also between 5 and 20 minutes after injection, parallel to the faster process 2

which dilutes the activity in the plasma, presumably by mixing the phosphate of the plasma with that of the second phosphate pool. According to this assumption, the decrease in plasma activity may be formulated as follows:—

$$-\frac{d\beta}{dt} = \frac{r}{P_1} (\beta - \lambda) + k\beta \quad (1)$$

In this equation

β = specific activity in plasma, expressed as activity per liter plasma in per cent of total injected dose assuming constant phosphorus content in plasma.

λ = specific activity in second pool, expressed as per cent activity per liter of fluid of that pool assumed to have same constant phosphorus concentration as plasma.

r = mixing rate (liters plasma per minute)

P_1 = plasma volume, liters.

k = relative rate of turnover of process 3.

To express λ in terms of β one may assume that at any time during the first hour after injection the total injected dose is distributed among blood plasma, (βP_1), second pool (λP_2), and a part removed from first and second pool by the first order process 3. At time, t , this third part amounts to

$$k \int_0^t \beta dt$$

Expressing the activities in per cent of the total injected dose one may thus write

$$\beta P_1 + \lambda P_2 + k \int_0^t \beta dt = 100 \quad (2)$$

or

$$\lambda = \frac{100 - \beta P_1 - k \int_0^t \beta dt}{P_2}$$

P_2 stands for the capacity of the second phosphate pool. This pool may be represented by the volume of a body fluid that has the same constant phosphorus concentration as the plasma and whose phosphate is in rapid exchange with the plasma.

Introduction of the result of Equation 2 into Equation 1 leads to the following expression:

$$-\frac{d\beta}{dt} = \frac{r}{P_1} \left(\beta - \frac{100 - \beta P_1 - k \int_0^t \beta dt}{P_2} \right) + k\beta \quad (3)$$

from which the turnover time t_t may be derived as follows:—

$$t_t = \frac{P_1}{r} = \frac{\beta - \frac{1}{P_2} \left(100 - \beta P_1 - k \int_0^t \beta dt \right)}{-\frac{d\beta}{dt} - k\beta} \quad (4)$$

The capacity P_2 of pool 2 is unknown. To estimate it, we have calculated for various times after injection the turnover time t_t on the basis of Equation 4 using various arbitrarily chosen ratios of $\frac{P_2}{P_1}$ from 4 to 19. For trial VII the turnover time was most nearly constant, during the period from 5 to 20 minutes after injection, when pool 2 had a capacity of 8 times the phosphate in the plasma. For trial VIII the best fitting pool ratio $\frac{P_2}{P_1}$ was 5.

The turnover time, time in which the equivalent of the phosphate in plasma is exchanged with the second pool—calculated by using the best fitting pool ratio—was 18 minutes for trial VII and 13 minutes for trial VIII.

Deviation from Prediction.—The term $k \int_0^t \beta dt$ in Equation 3 is small in comparison to $100 - \beta P_1$. Neglecting this integral the equation may be simplified to

$$-\frac{d\beta}{dt} = \frac{r}{P_1} \left(\beta - \frac{100 - \beta P_1}{P_2} \right) + k\beta \quad (5)$$

This equation may be integrated to

$$\beta = \frac{100}{P_1 + P_2} + \left(\beta_0 - \frac{100}{P_1 + P_2} \right) e^{-\frac{r}{P_1} \frac{(P_1 + P_2)}{P_2} (t - t_0)} \quad (6)$$

P_2 signifies the phosphate capacity of pool 2 expressed as liters liquid with the phosphate concentration of plasma. The other terms are defined with Equation 1. Fig. 1 shows the curves calculated according to Equation 6. For each of the two trials the standard deviation between a measured result and the predicted value amounts to ± 0.03 unit of the chart. This corresponds to a coefficient of variation of about 3 per cent of the mean result.

Process 2 When in Series with Process 3.—The calculations just described were based on the assumption that the first order decrease of plasma activity from 20 minutes to 1 hour after injection proceeds earlier parallel to the mixing process of pools 1 and 2. We have carried out also a series of calculations based on the alternate assumption that process 3 goes on in series instead of parallel to process 2. In this case, the rate of decrease may be formulated as follows:—

$$-\frac{d\beta}{dt} = \frac{r}{P_1} (\beta - \lambda) + k\lambda \quad (7)$$

The turnover time in this case becomes

$$t_i = \frac{P_1}{r} = \frac{\beta - \frac{1}{P_2} \left(100 - \beta P_1 - k \int_0^t \frac{100 - \beta P_1}{P_2} dt \right)}{-\frac{d\beta}{dt} - \frac{k}{P_2} \left(100 - \beta P_1 - k \int_0^t \frac{100 - \beta P_1}{P_2} dt \right)} \quad (8)$$

This calculation leads to turnover times which are about 20 per cent smaller than those resulting from the calculations based on parallelism between process 2 and 3. (See Table I.)

TABLE I
Capacity and Mixing Time in Mobile Phosphate Pool of Dairy Cows 8 Months after Calving

Trial No.	Cow No.	Body weight	Age	Milk yield per day	Plasma volume (measured)	Phosphorus content in plasma	Capacity ratio: Pool 2 plasma	Turn-over time of process 3	Mixing time of process 2	
									(a)*	(b)†
		kg.	yrs.	liters	liters	gm.		min.	min.	min.
VII	8-90	376	3	8.5	17.3	2.6	8	53	18	14
VIII	7-98	404	7.5	10.5	26.2	2.6	5	50	13	10

* (a), assuming that process 3 is parallel to mixing process 2.

† (b), assuming that process 3 is in series with mixing process 2.

Mixing Time of Process 2 in Other Cows.—Applying the mean pool ratio of $\frac{P_2}{P_1} = 6.5$ to 3 other cows injected with P^{32} and estimating their plasma volume as 5.6 liters plasma per 100 kilos body weight, we find for lactating cows (5 results) a mean turnover time of 14 ± 2 minutes and for two dry cows a turnover time of 21 ± 2 minutes.

DISCUSSION

For the kinetic analysis of the P^{32} concentration in plasma phosphate as a function of time after injection of the tracer, we have assumed that during the 20 minute period after injection the decrease of the tracer concentration in plasma is caused by two processes. The slower of these processes, designated as process 3, appears as a first order reaction between 20 minutes and 1 hour after injection, since during this period the logarithms of plasma activity are a linear function of time (see Fig. 2). The contribution of process 3 to the decrease of activity during the 20 minute period after injection may be calculated. The major part of the decrease of the tracer concentration in plasma during this period results from a process which is not of first order since the logarithms of activity during this period deviate consistently, if slightly, from a straight line (see Fig. 2).

Process 2 may be interpreted as the mixing of plasma phosphate with the

phosphate of a reservoir designated as the second pool. The relative mixing rate, defined as the part of the plasma phosphate which is exchanged with the phosphate of the second pool per minute, depends on the ratio of the phosphate content of the plasma and the corresponding phosphate capacity of the second pool. According to the fundamental concept of tracer technique, this mixing rate should not be influenced by the tracer. It should therefore be independent of time after injection. Based on this criterion, we postulate the capacity of the second pool as a constant in our calculation which makes the relative mixing rate, or its reciprocal, the mixing time, most nearly independent of time after injection.

A physiological significance may be found for the postulated second phosphate pool. The capacity of this pool is on the average for our two trials 6.5 times the phosphate content of the plasma. Assuming for the second pool the same phosphate concentration as that in plasma, this second pool might be thought of as a liquid 6.5 times the volume of the blood plasma and in rapid interchange with it. There is a liquid in the body in rapid exchange with the blood plasma that is known as the extracellular or interstitial tissue fluid. According to Gregersen,⁹ the volume of interstitial water amounts to about 5 times the plasma volume.

We may therefore assume that the second phosphate pool is mainly the phosphate in the interstitial body fluid. This working hypothesis accounts only for two-thirds to five-sixths of the second pool. Some of the difference may be explained by rapid early exchange of phosphate in parts of the body other than the interstitial tissue fluid.

The younger cow 8-90 contained on the average 15 mg. P per 100 ml. plasma; the older cow 10 mg. P per 100 ml. plasma. The total amount of plasma phosphorus is the same for both cows, namely 2.6 gm. According to the capacity ratios (pool 2 to pool 1) of 8 and 5 respectively, the second pool of cow 8-90 amounted to 20.8 gm. P; that of cow 7-98 to 13.0 gm. P. The P content of the entire body may be estimated from figures given by Hogan and Nierman¹⁰ for Hereford steers which presumably are applicable enough to our cows to give an approximate estimate of the magnitude of the mobile phosphate pool in terms of the phosphate content of the whole body. The "mobile pool" (plasma phosphorus plus second pool) amounted to 23.4 gm. P, or 0.94 per cent of the P in cow 8-90. The mobile pool of the older cow 7-79 was 15.6 gm. P, or 0.54 per cent of the P presumably in her body.

To test our working hypothesis identifying the second phosphate pool with the phosphate in the interstitial tissue liquid will require a good deal of experimental work. That is also true for testing the two suggestions: (a) that the

⁹ Gregersen, M. I., in MacLeod's *Physiology in Modern Medicine*, (P. Bard, editor), St. Louis, The C. V. Mosby Company, 9th edition, 1941, 1065.

¹⁰ Hogan, A. G., and Nierman, J. L., *Univ. Missouri Research Bull.* 107, 1927, 42.

capacity ratio of the second pool to plasma phosphate is greater in younger animals, (b) that the rate of mixing phosphate between plasma and the second pool is greater for lactating cows than for dry cows.

For studying the kinetics of slower phosphate exchange processes in the animal body the knowledge of the mixing rate of the plasma phosphate with the second pool and the capacity of that pool is of obvious importance. The turnover rates of slow processes of exchange with the plasma phosphate could probably be expressed more clearly in terms of the "total mobile pool," that is the "phosphate in plasma plus second pool," than in terms of plasma phosphate alone.

The deviation of the early decrease of P^{32} concentration in plasma from the behavior of a first order process is ascribed here to the limited capacity of the second pool. A similar treatment may be applicable to a considerable number of other exchange processes in the animal body.

The greater P_2 , in comparison to P_1 , the more Equation 3 approaches that of a first order reaction, namely

$$-\frac{d\beta}{dt} = \left(\frac{r}{P_1} + k\right)\beta$$

since λ in Equation 1 approaches zero when $\frac{P_2}{P_1}$ approaches infinity. For an unlimited capacity of the second pool equation 6 becomes

$$\beta = \beta_0 e^{-(r/P_1)(t-t_0)}$$

which aside from different terminology, is the same as Equation 1 of Zilversmit and his coworkers.¹¹

SUMMARY

Radiophosphate was injected into the left jugular vein of dairy cows. Blood samples were taken frequently from the right jugular vein during the first hour after injection. Between 20 minutes and 1 hour after injection, the decrease in plasma radioactivity could be formulated as a first order process, designated as "process 3," with a turnover time of 50 minutes. From 5 to 20 minutes after injection the decrease in plasma activity could be interpreted as the result of mixing plasma phosphate with another phosphate pool, designated as the second pool. The capacity of this second pool was derived as a constant in a kinetic equation, so chosen that the resulting mixing rates were independent of time.

For two cows the capacity of the second pool was 5 and 8 times, respectively, the phosphate content of the plasma. This result led to the working hypothesis that the major part of the second pool was the phosphate in the interstitial tissue fluid.

¹¹ Zilversmit, D. B., Entenman, C., Fishler, M. C., and Chaikoff, I. L., *J. Gen. Physiol.*, 1942, **26**, 335.

The turnover time of the plasma phosphate in the mixing process with the second pool amounted to an average of 14 minutes for 5 lactating cows, and an average of 21 minutes for 2 dry cows. This result was obtained under the assumption that the slow first order process 3 is parallel to the mixing process. The assumption that the slower first order process is in series with the mixing process reduces the resulting mixing time to about four-fifths of that reported above. The calculation of process 2 which deviates from first order may be applicable to numerous turnover processes in which both exchange pools have a limited capacity.