QUANTITATION OF P METABOLISM IN THE RABBIT BY MEANS OF ^{32}P

J. M. VAN LEEUWEN*) and J. W. VAN RIEL*)

Summary

Cr_2O_3 was found to be very effective in correcting the daily variations in the excretion of dry matter, Ca, Mg, and P in rabbit faeces. It can shorten the length of a balance experiment by 50%. We have an idea that a considerable part (about 50%) of the total P retained is incorporated in the organism so quickly that it cannot be observed with the intravenous radioactive tracer method.

The excretion of the endogenous faecal P amounted to 20 mg per day. No significant difference was found between the apparent and the actual absorption coefficient of P.

The exchangeable P pool of the organism amounted to 690 mg. The experiment results should be subjected to detailed biometric evaluation.

Samenvatting

Cr_2O_3 blijkt goed bruikbaar voor correctie van variaties per dag in de uitscheiding van droge stof, Ca, Mg en P via de faeces van het konijn. De duur van de balansproef kan hierdoor met 50% worden bekort.

De indruk bestaat, dat een belangrijk deel (± 50%) van het totaal geretineerde P zo snel wordt ingebouwd in het organisme dat met de intraveneuze radio-actieve tracer methode dit niet wordt waargenomen.

De endogeen faecaal uitgescheiden hoeveelheid P bedroeg 20 mg per dag. Er bleek geen significant verschil tussen de schijnbare en werkelijke P-absorptiecoëfficiënt.

De uitwisselbare P-pool van het organisme bedroeg 690 mg. Een uitgebreide biometrische evaluatie van de proefgegevens is gewenst.

Introduction

The importance of more thorough P research is underlined by attempts to rationalise and restrict P feeding to livestock because of local surpluses of phosphorus originating in the manure, and hence a possible danger to the environment. One conceivable remedy would be to reduce the P level in the feed to below the accepted standard and then to stimulate P absorption artificially in such a way that the phosphorus requirements with respect to maintenance, growth, and production would still be met. A report on such an artificial corrective measure will be published in another connection (7).

The present study is concerned with two questions:

1. How can the daily variability in P excretion in the faeces be corrected so that reliable and reproducible data may be collected in a short experiment of less than 10 days? The variability itself is primarily related to changes in the rate at which P passes through the alimentary canal.
2. How are we to interpret the results obtained after oral or intravenous administration of sodium (^{32}P) orthophosphate ($Na_3{}^{32}PO_4$) to a healthy rabbit on a normal diet?

The investigators have based their search for the answers to these questions on the belief that the most reliable information would be obtained by a combination of the classical (chemical)

*) At the time of the experiment Dr. J. M. van Leeuwen and Mr. J. W. van Riel worked at the "Hoorn" Institute for Livestock Feeding and Nutrition Research. Dr. van Leeuwen is now attached to the Central Veterinary Institute, Rotterdam Branch, and Mr. van Riel to the Animal Physiology Laboratory, Amsterdam University.

balance method with the modern (physical) tracer method. The classical method alone provides an incomplete picture of absorption from the intestine and excretion in the faeces and urine. The endogenously excreted P fraction and the exchangeability of P in the P pool of the body are quantities which can be measured with a radioactive tracer but which clude observation by the old methods. This new information increases our knowledge of the physiology of P.

Set-up and feeding

The concentrated feed consisted of equal parts of coconut flakes, linseed meal, barley meal, and maize meal, to which extra minerals (1.5% $CaCO_3$, 0.4% NaCl, traces of $CuSO_4$ and $CoSO_4$, but no extra phosphate) and chromium oxide had been added. The total dry feed mixture contained 0.54% P, 0.81% Ca and 0.50% Cr_2O_3. The animals each received 80 g of this feed per day, mixed with 140 ml water to form a thick mash. The amount of water they drank was measured.

The animals were kept in wire cages; their faeces and urine were collected separately (7).

Two rabbits were used for the chromium oxide correction experiment (in August 1971), and four for the isotope experiment (in October 1972); all were Viennese rabbits, does, and nearly adult.

The chromium oxide correction experiment covered a preliminary period of 11 days, followed immediately by a 10-day experimental period.

During both periods each animal received 400 mg Cr_2O_3 a day, mixed in its food, and daily balances were kept. In the faeces collected during the preliminary period, only Cr_2O_3 was determined, while in the faeces collected during the experimental period Ca, P, and Mg were also determined.

The isotope experiment involved a changeover: two rabbits each received 84 μCi ^{32}P in the form of $Na_3{}^{32}PO_4$ mixed in their food one on day 0, and two other rabbits were administered the same amount of ^{32}P intravenously (into a vein in the ear) at the same time at the start of the experiment. After a week the treatments were reversed. The results of both treatments could be compared within one group of 4 animals by plotting startpoint and change-over time to the same moment. Observations, which consisted of analysis of blood, urine and faeces samples, were stopped after a fortnight.

Analyses

Chemical analyses were made by means of standard "Hoorn" methods, as described in other publications (Annual Reports of the "Hoorn" Institute for Livestock Feeding and Nutrition Research).

For the determination of ^{32}P, the Cerenkov effect in an aqueous medium was used (4). Measurements were carried out with a Beckman Liquid Scintillation Counter (LS-150). To calculate efficiency, specific activity, and balance results, a computer belonging to the Agricultural University, Wageningen, was used.

Notation

The rate of change of the specific activity of ^{32}P in blood plasma (R_s) is represented by the formula (4):

$$R_s = A_1 e^{-a_1 t} + A_2 e^{-a_2 t} + A_3 e^{-a_3 t}$$

The endogenous faecal P fraction (v_f) is calculated by means of the following formula (1):

$$R_F\Big]_{48}^{120} = v_f \int_{48}^{120} f(t)dt,$$

in which $R_F\Big]_{48}^{120}$ is the quantity of ^{32}P excreted in the faeces between 48 and 120 hours after the injection of ^{32}P. A check on the result of v_f is obtained by means of the following equation, t_1 being 48 hours and t_2 being 120 hours:

$$\frac{^{32}P \text{ in urine}}{P \text{ in urine}} = \frac{^{32}P \text{ in faeces}}{v_f}$$

This check gives very satisfactory results.

The pool of exchangeable phosphorus and the quantity of phosphorus absorbed from the pool per time unit (v_{o+}) in the organism is calculated by means of the following formula (1):

$$R_t\Big]_o^t = P.f(t) + v_{o+} \int_o^t f(t)dt,$$

in which $R_t]_o^t$ is the total quantity of ^{32}P present in the body for instance at t_1 = 48 hours and t_2 = 120 hours after injection. This produces two simultaneous equations with two unknowns. The quantity of P absorbed per time units (v_a) is calculated by means of the formula:

$$v_a = v_i - v_F + v_f$$

The meaning of these and subsequent abbreviations is given in Table 3.
The actual absorption coefficient of P, (α), can be obtained as follows:

$$\alpha = \frac{v_a}{v_i}$$

The apparent absorption coefficient of P, (α^1), equals:

$$\alpha^1 = \frac{v_i - v_F}{v_i}$$

The balance yields $\Delta = v_i - (v_u + v_F)$
The endogenous excretion of P in the intestine (v_d) is calculated by means of the following equation:

$$v_f = v_d(1 - \alpha).$$

Results

1. Use of Cr_2O_3 to correct the daily variability in P excretion in the faeces

Table 1 shows that correction by Cr_2O_3 reduces by 3 to 5 times the daily variability in the excretion of the faeces, on the basis of air-dry matter, and of the quantities of P, Ca and Mg excreted in the faeces.

It can be seen from Figure 1 that the duration of a P balance can be shortened by 4 to 6 days (on average of 5 days) with the Cr_2O_3 corrective. Without correction the experiment would have to last more than 10 days to produce reasonably constant results. These conclusions apply also to Ca, Mg, and air-dry matter, but this aspect will not be dealt with further in this paper. The correction is obtained by multiplication of the quantity actually excreted by the factor $\frac{C_2O_3 \text{ in the food}}{C_2O_3 \text{ in the faeces}}$ on the basis of equal units of weight per unit of time.
The reliability of the Cr_2O_3 corrective is partly explained by the high recovery of Cr_2O_3 (100.5% on average) after an 11-day preliminary or adaptation period (Table 2).
Figure 2 shows that a constant excretion level of Cr_2O_3 in the faeces is achieved after only 6 days.

2. Results obtained after oral and intravenous administration of Na_3PO_4 to healthy rabbits on a normal diet

Figure 3 represents the average rate of change of radioactive ^{32}P in the blood plasma of four rabbits over a period of 340 hours after oral or intravenous administration of 84 μCi $Na_3{}^{32}PO_4$. The specific activity of ^{32}P as a percentage of the dose is shown logarith-

Table 1. Variation coefficients (%) of the daily excretion of air-dry matter, P, Ca, and Mg in the faeces of rabbits I and II during a 10-day experiment.

	air-dry matter		P		Ca		Mg	
	I	II	I	II	I	II	I	II
without Cr_2O_3 sorrective	16.5	29.2	16.9	30.8	16.3	29.5	16.7	30.4
with Cr_2O_3 corrective	3.3	6.4	5.7	6.1	5.3	6.1	4.3	6.2

Table 2. Cr_2O_3 excreted in the faeces by rabbits I and II, in % of the intake.

	rabbit		
	I	II	x
11day preliminary period	89.6	72.3	81.0
10-day experimental period	103.7	97.4	100.5

Table 3.

Chief parameters of the P metabolism of four young rabbits calculated per rabbit per day.

v_i	= P intake in food (mg)	428
v_p	= P excretion in faeces (mg)	341
v_u	= P excretion in urine (mg)	25
α'	= apparent absorption coefficient of P (%)	20[a]
α	= actual absorption coefficient of P (%)	25[a]
v_{a_1}	= P absorption from food (mg)	107[b]
v_{a_2}	= P reabsorption after absorption, circulation and endogenous excretion (mg)	7
v_d	= endogenous P excretion in intestine (mg)	27
v_f	= endogenous P excretion in faeces (mg)	20
Δ	= total P retention by the organism (= P balance) (mg)	62[c]
v_{o+}	= P retention in the organism, measurable by blood ^{32}P (mg)	29[c]
$v_T = v_f + v_u + v_{o+}$	= measurable P excretion from exchangeable P pool (mg)	74
P	= magnitude of exchangeable P pool (mg)	690

a. The difference is insignificant; b. Variation coefficient = 12%;

c. The difference is significant $P < 0.05$.

mically on the y-axis. It is noticeable that after 50 hours already the decay curves are running parallel. Before that time the decan of $_{32}P$ in the blood-plasma after oral administration is found to become constant approximately one day earlier than after injection. Possibly inorganic ^{32}P is "incorporated" into the organism through the intestinal wall more quickly than when it is injected intravenously into the bloodstream. Figures 4 and 5 represent the cumulative excretions in faeces and urine of ^{32}P as percentages of the dose. These figures show that 14 days after oral administration 69% of the initial dose of ^{32}P had left the rabbits' bodies; the corresponding figure after injection was 43%. At that point the rabbits that had received an injection contained nearly twice as much radioactivity as those that had received an oral dose of the same quantity of ^{32}P.

Table 3 shows the results of the experiment in which ^{32}P was administered intravenously to four rabbits, together with the balance data.

The experiment in which ^{32}P was administered orally was not taken into account in the calculation of the parameters in Table 3. Figure 6 is a chematic representation of the results given in Table 3.

Figure 7 shows how the inorganic P content of blood plasma varies in a single rabbit after blood samples have been taken at different intervals (minutes and hours). This observation was not made during the isotope experiments.

Discussion

1. Since the isotope experiments described above should preferably be completed within just a few days because of the swift biological and radioactive decay of the isotope, it is absolutely essential to use a means of correcting

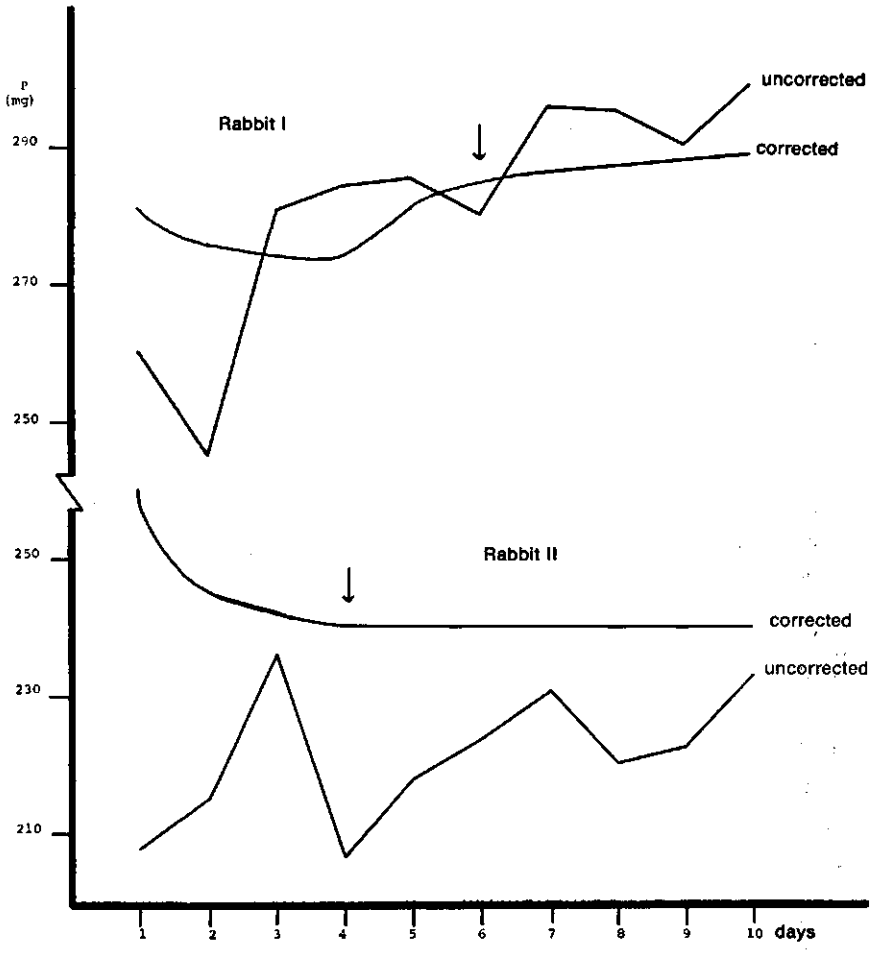


Fig. 1. Effect of Cr_2O_3 correction on the average excretion of P per animal per dag in the faeces of two rabbits, represented per balance period of increasing number of days (from 1 to 10).

the daily variations in the excretion of minerals in the faeces and urine.

Once a good corrective such as Cr_2O_3 has been found, it is vital that an adequate preliminary period (at least 6 days) should be allowed, so that constant and maximum recovery (100% in this case) is achieved. Although the need for this correction is self-evident, it is in fact very often omitted; this can lead to seriously inaccurate results.

2. Figure 3 shows that oral administration of ^{32}P results more quickly in a constant decay of the specific activity in blood plasma than does intravenous injection. Nonetheless, the oral isotope experiment was not taken into account because of the lower level of radioactivity in the blood, and because it may be assumed that the absorption coefficient of inorganic P cannot be compared with that of organic P in the food. In the intravenous injection experiment a very small quantity of inorganic radioactive P is injected into the blood, which normally contains an excess already of inorganic nonradioactive phosphorus.

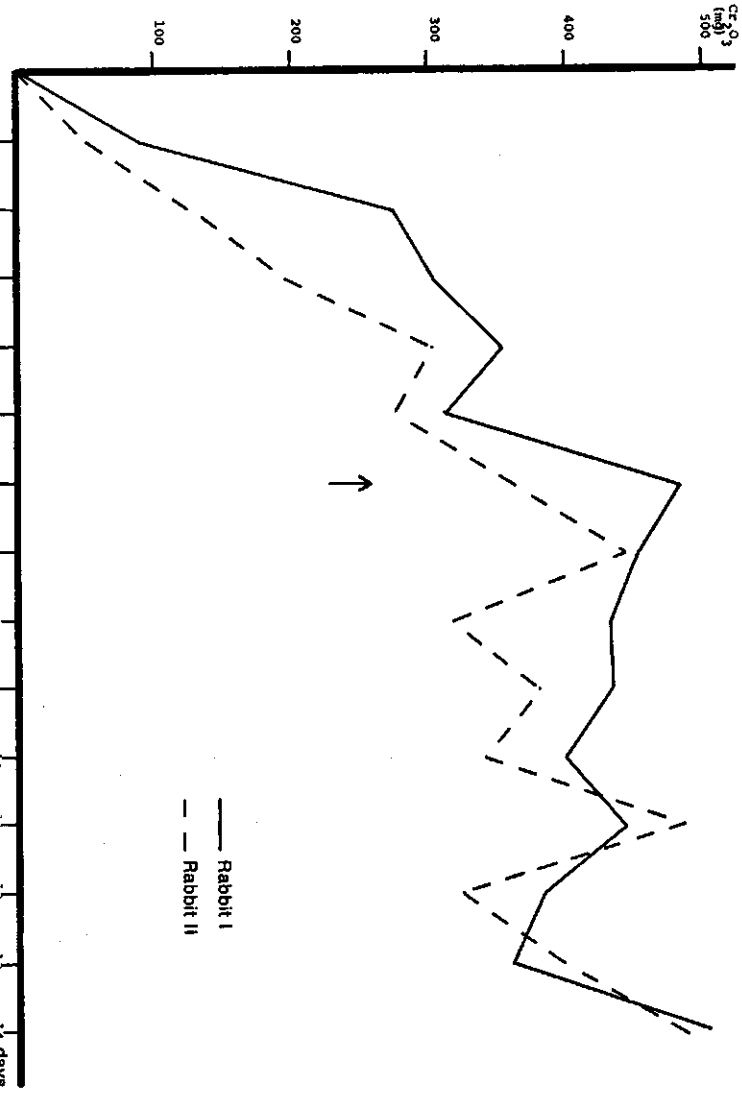


Fig. 2. Excretion pattern of Cr_2O_3 in the faeces of two rabbits receiving 400 mg Cr_2O_3 per day in their feed.

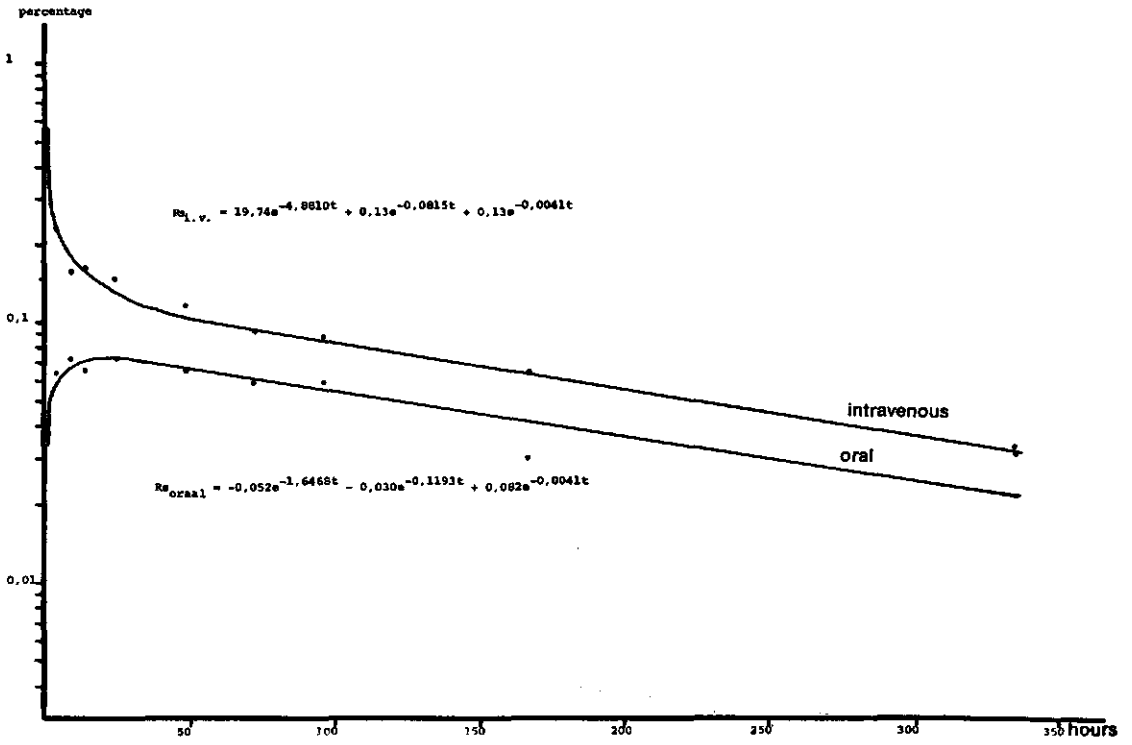
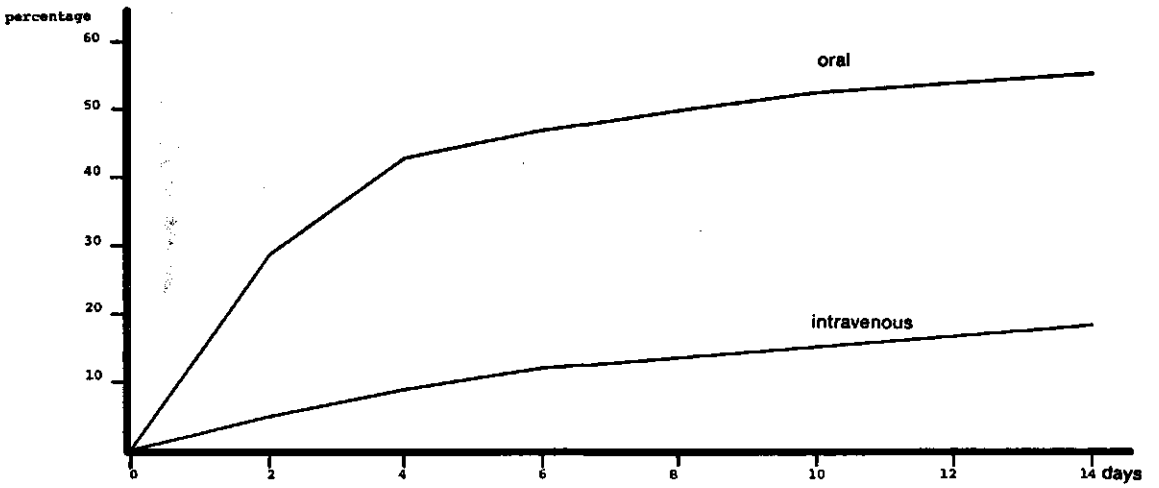


Fig. 3. Average rate of change of the specific activity (Rs) of ^{32}P in the blood plasma of four rabbits over a period of 340 hours after oral and intravenous administration of equal quantities of $Na_3{}^{32}PO_4$ (R in % of the dose).

Fig. 4. Cumulative excretion of ^{32}P (in % of the dose) in the faeces after oral and intravenous administration of $Na_3{}^{32}PO_4$.



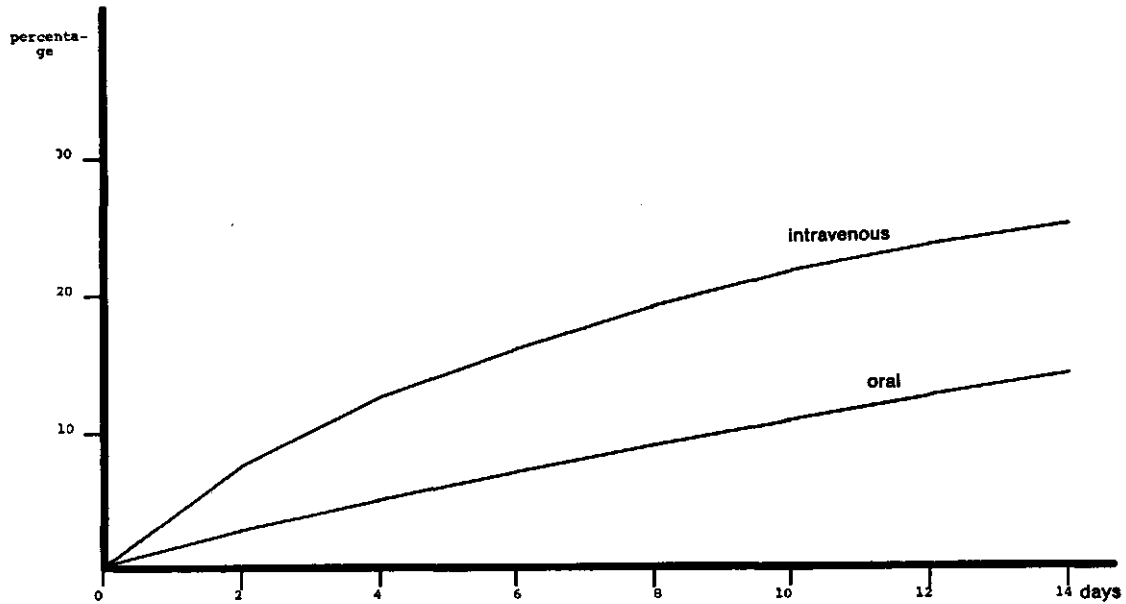


Fig. 5. Cumulative excretion of ^{32}P (in % of the dose) in the urine after oral or intravenous administration of $Na_3{}^{32}PO_4$.

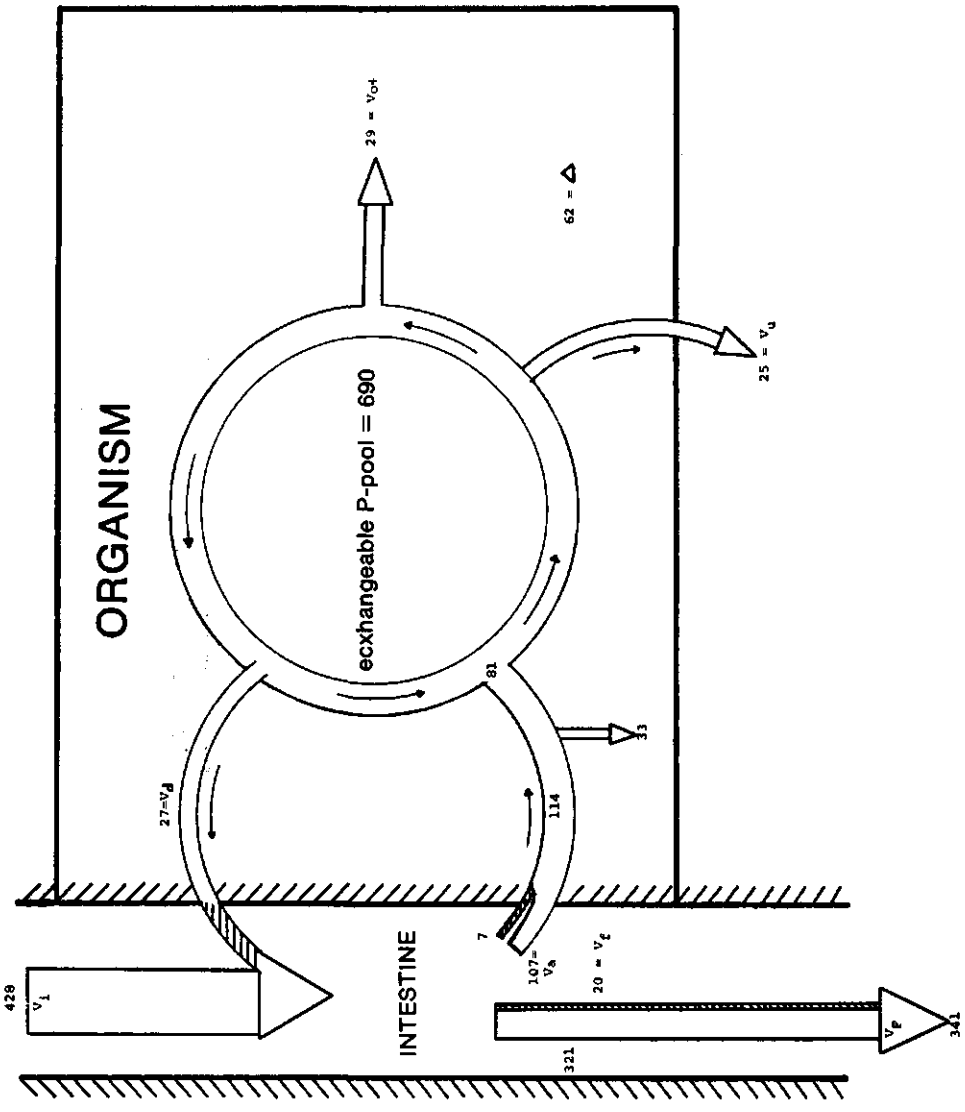


Fig. 6. Schematic representation of P metabolism in the rabbit (mg per rabbit per day). See Table 3 for the meanings of abbreviations.

This provides a reliable experimental model. The fact that unknown factors, such as stress, may cause fluctuations in the inorganic phosphorus content of blood plasma (Figure 7) does not prevent the decay curve of the specific activity of ^{32}P in the blood plasma from having a constant rate of change (Figure 3). This is understandable if it is realised that specific activity is the ratio between radioactive and nonradioactive P. Since the above fluctuation quickly dies away, it is probably of only slight significance for the evaluation of the other results. To make certain however, blood samples should not be taken too frequently at the beginning of the isotope experiment.

This experiment also shows that there is no significant difference between the apparent and the actual absorption coefficient. The endogenous faecal

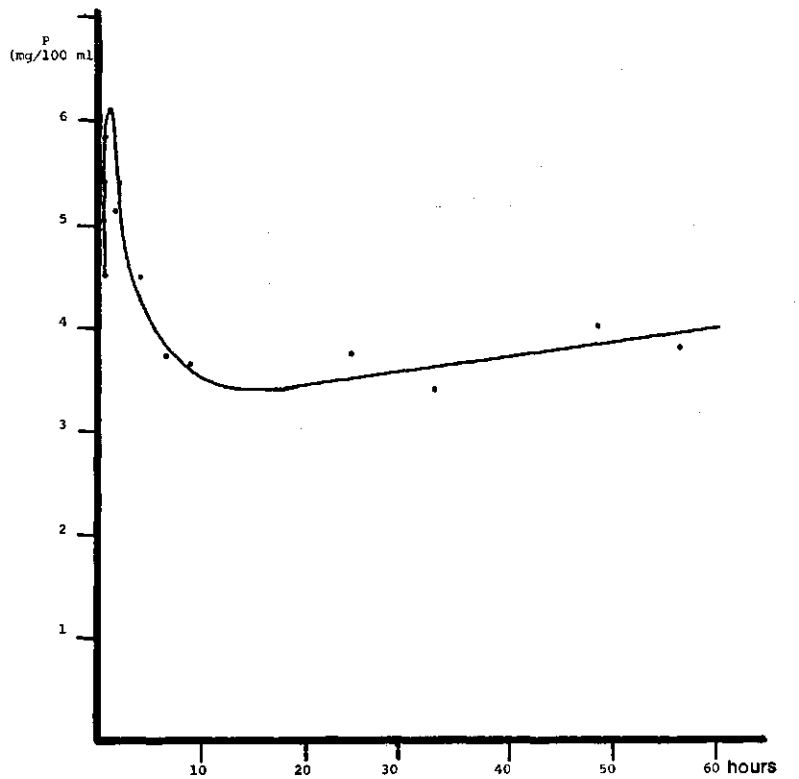


Fig. 7. Fluctuations in the inorganic P content of blood plasma in the rabbit, blood samples taken at various intervals.

fraction (v_f) in fact constitutes hardly 5% of the total P intake (v_i).

Part of the total P retention was found not to be measurable by the isotope method (the difference between Δ and $v_{o+} = 33 \pm 8$ mg). This can be explained by the direct retention of P absorbed in the intestinal wall and other internal organs, partly outside the peripheral circulation.

For a precise biometric evaluation of the experiment results, the method of calculation used by us must be compared with other methods based, for instance, on compartment systems (1, 5, 6), which are evolved by computers using simulation techniques. To do so would far exceed the scope of this article. Our method of calculation is a slightly modified version of that of Bauer *et al.* (2, 3). It is simple and capable of a physiological interpretation. The compartment model is difficult to interpret in physiological terms, especially in respect of P metabolism, since P has a general function and occurs widely in the organism.

REEERENCES

1. Aubert, J. P., Bronner, F., and Richelle, L. J.: Quantitation of Calcium metabolism. Theory. *J. Clin. Invest.*, 42, *885, (1963).*
2. Bauer, G. C. H., Carlsson, A., and Lindquist, B.: Evaluation of Accretion, Resorption, and Exchange Reaction in the Skeleton. *Kgl. Fysiograf. Sällskap. Lund Förh.*, 25, *1, (1955).*
3. Bauer, G. C. H., Carlsson, A., and Lindquist, B.: Bone Salt Formation Measured in Infants by Means of P^{32}. *Acta Paediatrica*, 44, *477, (1955).*
4. Comar, C. L.: Radioisotopes in Biology and Agriculture. McGraw-Hill Book Comp., New York, (1955).
5. Kment, A., Leibetseder, J., and Skalicky, M.: Untersuchungen zum Phosphor-Stoffwechsel des Kaninchens unter Verwendung der Cerenkov-Strahlung von ^{32}P. *Zentralbl. für. Vet. Med. Beiheft 11 Symp. Hannover, (1968).*
6. Krawielitzki, K.: Untersuchungen zum Multikompartmentsystem des Phosphat-Stoffwechsels mit Hilfe von Radiophosphat. *Wiss. Zschr. Univ. Rostock*, 18, *955, (1969).*
7. Leeuwen, J. M. van, and Riel, J. W. van: De invloed van zoutzuur op absorptie en retentie van Ca, P en Mg bij het konijn. I.V.V.O. "Hoorn". Rapport no. 103.