CALCIUM AND PHOSPHORUS METABOLISM IN GROWING PIGS STUDIED BY THE BALANCE TECHNIQUE AND SIMULTANEOUS RADIO-CALCIUM AND RADIO-PHOSPHORUS KINETICS

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SUMMARY

Balance experiments and kinetic studies of Ca and P were performed with 18 growing pigs representing two weight classes (35 kg and 65 kg) and fed varying amounts of Ca and P.

Balance studies showed that net absorption and mineral retention declines relatively with increasing intake, thus evidencing a homeostatic regulation of mineral utilization.

Calcium absorption and retention was regulated solely by the intestinal processes while P retention was also moderated by urinary excretion.

Kinetic studies revealed that the intestinal input of mineral was regulated by bone resorption. Bone accretion was found to be constant and independent of the level of absorption. Bone resorption on the other hand, declined with increasing mineral input, thereby regulating the net mineral retention.

The live weight of the pigs did not influence the absorption capacity significantly, but pigs of 65 kg LW showed a distinct tendency to a higher absorption than the smaller pigs.

Accretion rates were higher for the larger pigs, but the higher accretion was due to the larger bone mass. Plateau values of Ca-balance in relation to intake (g/day) were remarkably similar to bone accretion, thus providing a simpler method for estimating bone accretion.

The implication of these findings in relation to traditional methods for assessment of mineral requirements are discussed. It is proposed that first priority should be given to identification and definition of criteria for assessment of adequate bone growth and development in relation to production.

Factors such as retention and availability or efficiency of utilization are determined by the pigs' physiology and should therefore be included in the assessment of requirements.

Evaluation of mineral sources' adequacy to supply minerals can thus be reduced to the assessment of the mineral's solubility in the particular source.

INTRODUCTION

The metabolism of Ca and P is tightly regulated by a hormonal system constituted by the parathyroid hormone, calcitonin and 1,25-(OH)2D3, (DeLuca 1979).

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The combined simultaneous action of the system on entrance (intestinal absorption and bone resorption) and escape (deposition and excretion) routes maintains the blood concentration of these minerals at a level just enough to permit bone mineralization to proceed (Parfitt 1976; DeLuca 1979). This homeostatic control means that assessing the nutritional requirements of the animal and formulation of the optimal dietary supply to satisfy these requirements, is an integral problem which can not be solved adequately by attending the issues separately.

Nonetheless, the majority of experiments performed are devoted to either one of the topics (Agricultural Research Council (ARC) 1981) resulting in controversial results due to differences in experimental conditions, in the response criteria chosen or in the way in which experimental inputs/outputs are expressed.

With this background, a research project was started to elucidate digestion, absorption, and utilization of minerals in growing pigs in order to establish standardised methods for Ca and P supply evaluation and concomitantly the mineral requirements of pigs for optimum performance.

This report will deal with the results of Ca and P balance experiments and kinetic studies with the specific purpose of:

- assessing the quantitative relationship of mineral intake to absorption and utilization

  estimating bone accretion and resorption and evaluation of these parameters contribution to the definition of requirements or to identification of adequate response criteria.

MATERIALS AND METHODS

Three litters of six females each, were selected from the institute's herd and penned individually from 15 kg live weight to the start of the experiments. Three pigs of each litter were randomly allotted to one of three dietary Ca and P levels, given with a semi-synthetic basal diet, when the pigs were about 28 kg live weight. The remaining three pigs continued on a standard diet until about 55 kg where the same experimental conditions were imposed.

The pigs were fed the experimental diet for two weeks and were thereafter placed in stainless steel metabolic cages. After an adaptation period of 5 days, the pigs were sedated and indwelling catheters were placed in the right and left jugular vein for blood sampling and in the urinary bladder for urine collection. Twenty-four hours later, the pigs were given a single injection of 45CaCl2 and Orthophosphate-32P (about 0.6 MB/kg LW) and blood sampling started 1-3 minutes later. Blood samples were taken at increasing intervals (minutes) the first 3 hours, between several hours the first two days and once daily from then on.

Blood samples were centrifuged immediately after withdrawal, and plasma samples were obtained in duplicate for radioactivity and mineral content determinations, respectively. Faeces and urine were collected and assayed for radioactivity and mineral content daily for 8-10 days.

At the end of the experiment, the pigs were sacrificed and the right femur and the III and IV metacarpal of the left leg were extracted, cleaned for adherent soft tissue, and weighed.

Samples of basal diet, calcium carbonate, monosodium phosphate-7 H_2O faeces, and bones were dry ashed at 525°C for six hours and dissolved in concentrated HCl. Samples of plasma and urine were wet ashed in concentrated nitric acid and perchloric acid at 200°C for 4 hours.

Calcium was assayed in the ash solution by atomic absorption spectro-photometry; phosphorus was determined colorimetrically (420 nm) by the vanadomolybdate procedure.

Radioactivity was determined directly in plasma and urine samples, and in the ash solutions of faeces and bones by liquid scintillation counting. The radioactivity of 45Ca and 32P were separated and quench corrected by the external standard channel ratio method (Snipes and Lengeman, 1971). Parameters of Ca and P metabolism were estimated by the combined data of the balance experiment and the kinetic study.

Balance parameters included: mineral intake (VI), net absorption from the intestine (Va) total mineral excretion with faeces (VF), and balance (Ba) which was determined as the difference between daily intake and daily excretion (Ba = VI - VF - Vu).

Data on the specific activity in plasma of both isotopes was described by a 3-compartment model, as described by Aubert et *al.* (1963) and Besancon and Gueguen (1969).

The model adopted is shown in Fig. 1 (Aubert *et al.* 1963). Calculation of transport rates involves the assumption of a steady-state during the experiment. Mineral transport out of the exchangeable pool (VT) occurs by the endogenous faecal losses (Vf), urinary excretion (Vu) and mineral accretion by bones and soft tissues (Vo+). If steady-state conditions prevail, then outputs and inputs must be equal, hence the sum of total absorption from the intestine (VTa = VI - VF + Vf) and mineral removal from the non-exchangeable pool (Vo-) must equal VT.

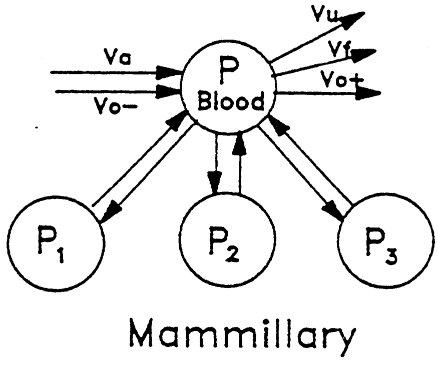

Fig. 1. Model of mineral exchange. (Mod. after Aubert *et* al. 1963), P: Control pool; Va: net absorption from the gut; Vo-: mineral resorption; Vu: urinary excretion; Vf: Endogenous faecal excretion; Vo+: bone accretion.

Faecal endogenous losses (Vf) was calculated as the ratio of the accumulated radioactivity (CPM) excretion with faeces and the integral of plasma specific activity (CPM/mg) in the same period of time.

Similarly, bone accretion was estimated as the ratio of the radioactivity amount (CPM) in bones and the integral of plasma specific activity (CPM/mg) from the time of injection to the end of the experiment.

Alternatively, bone accretion of Ca and P were also calculated by the BCL-formulation

(Bauer *et* al. 1957) which prescribes solution of two equations at two different time intervals. Equations in this study were defined at 4 and 7 days, respectively.

Resorption of both Ca and P were calculated from the bone data as the difference between bone accretion and mineral balance. Results were analysed for litter + experimental period effect and live weight effect by covariance analysis,with mineral input as the covariate.

RESULTS AND DISCUSSION

The relationship between absorption and intake is shown in Fig. 2 and 3 and between balance and intake in Fig. 4 and 5 for Ca and P, respectively. The function of absorption and balance was determined for each LW, as dependent on the natural logarithm of daily intake. The corresponding equations (1 to 8) are shown in Table 1.

The pattern of Ca retention (Ba) in relation to intake is similar to that found by Mudd *et al.* (1969) and Schenkel and Müller (1984) in pigs and with Sammon *et* al. (1970) in rats. The consistent curvilinear relationship of Va and Ba with intake as reflected by Fig. 2 - 5 and the eqs. (1-8) is in good agreement with the general view that Ca absorption involves active transport and therefore is subjected to regulation as demonstrated by Sommerville *et al.* (1985) in pigs and Bronner (1987) in rats.

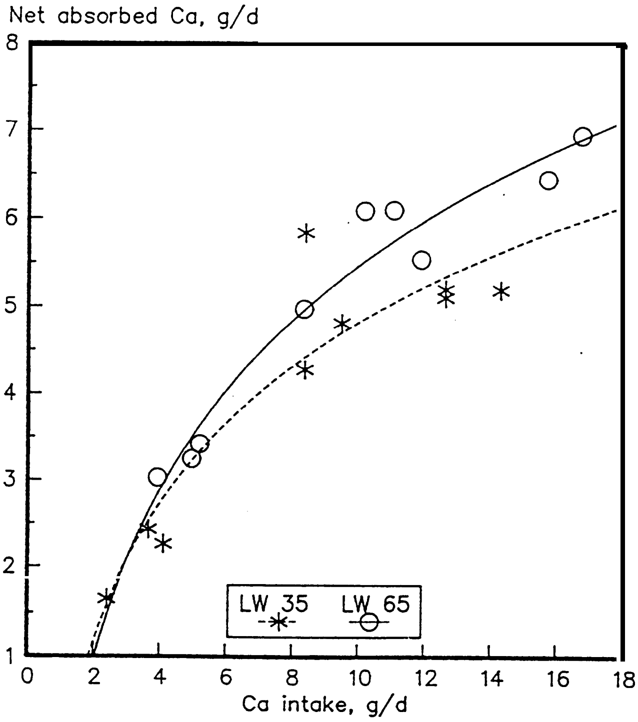

Fig. 2. The influence of intake and LW on net absorption of Ca

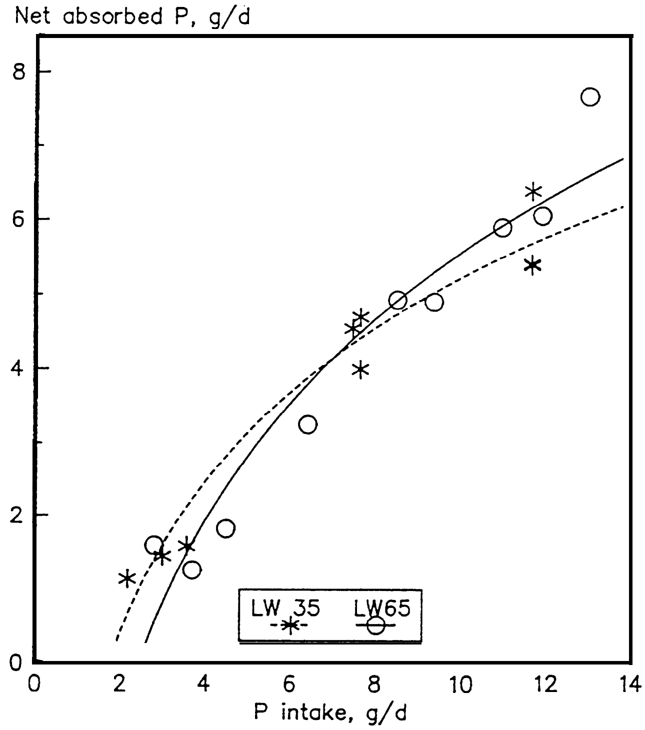

Fig. 3. The influence of intake and LW on net absorption of P

Even when the pattern of absorption and retention of Ca and P seems similar, some basic differences are noteworthy. In the first place, the excretion of Ca with the urine is negligible, except for the lowest levels of intake, which can be shown to be due to insufficient dietary P (Pointillart *et al.* 1986) indicating that absorption and ultimately the retention of Ca is regulated solely through the intestine, as evidenced by the similarity between the equations of Ca absorption and retention. Urinary excretion of P, on the other hand, increased with increasing intake which implies that the utilization of consumed P is also regulated by renal action.

Generally, results on Va and Ba for both Ca and P tended to indicate a higher capability of absorption and retention by the larger pigs. These differences were in no case significant. However, these results negate the common generalized concept of a declining' absorption potential with age (ARC 1981), which probably might apply if weaners, adult, and ageing animals are compared, but not necessarily if the comparison is made within an interval of the growing period of the pig, as found by Vemmer (1982).

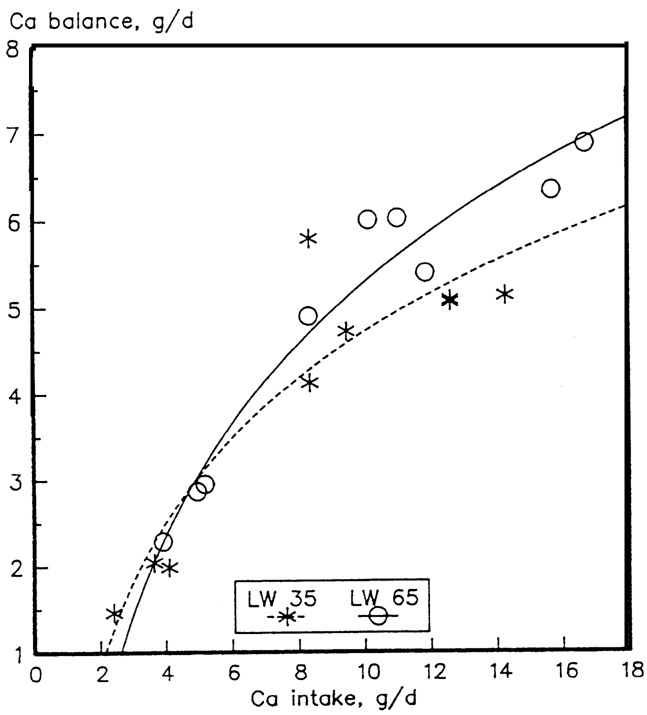

Fig. 4. The influence of intake and LW on Ca balance

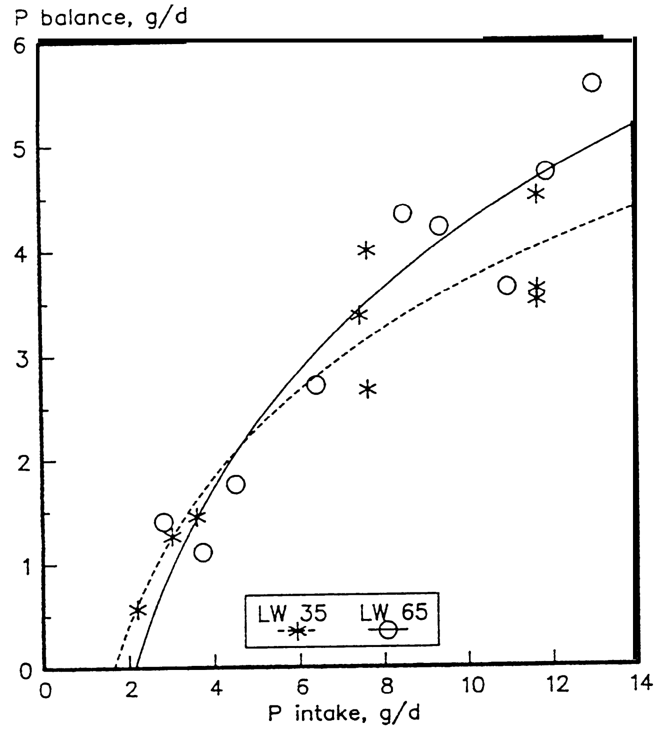

Fig. 5. The inflence of intake and LW on P balance

Table 1. The regression of net absorption (Va) and balance (Ba) on the natural logarithm of daily intake (lnVa) of Ca and P in pigs of 35 kg and 65 kg live weight (LW)

Mineral	LW	Eq. no.	Variable	Intercept	b	r^2
Ca	35	1	Va	-0.4	2.3	0.86
Ca	65	2	Va	-1.0	2.8	0.95
Ca	35	3	Ba	-0.8	2.4	0.86
Ca	65	4	Ba	-2.1	3.2	0.95
P	35	5	Va	-1.8	3.0	0.95
P	65	6	Va	-3.7	4.0	0.92
P	35	7	Ba	-1.0	2.0	0.89
P	65	8	Ba	-2.1	2.7	0.89

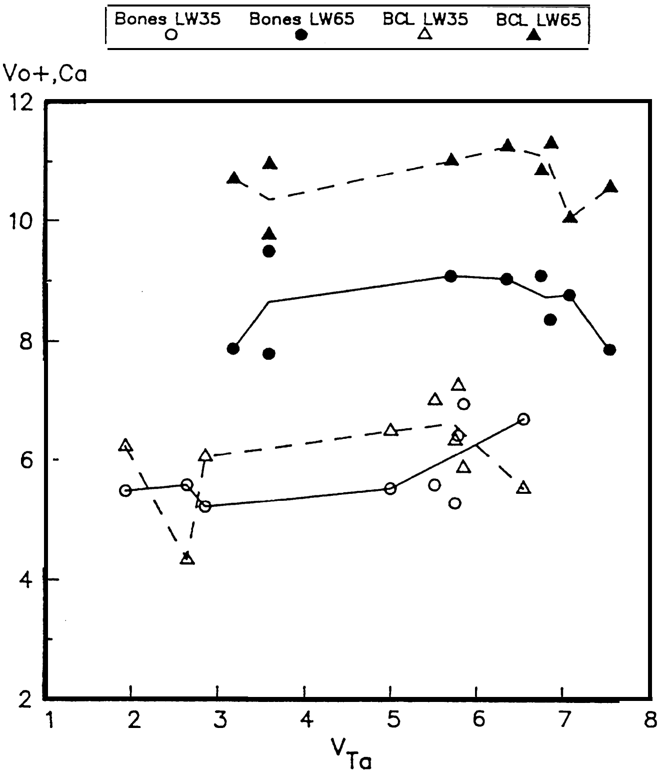

Fig. 6. Ca accretion (Vo+) estimated by bone radioactivity and the BCL-formulation in relation to live weight (LW) and total mineral absorption (VTa)

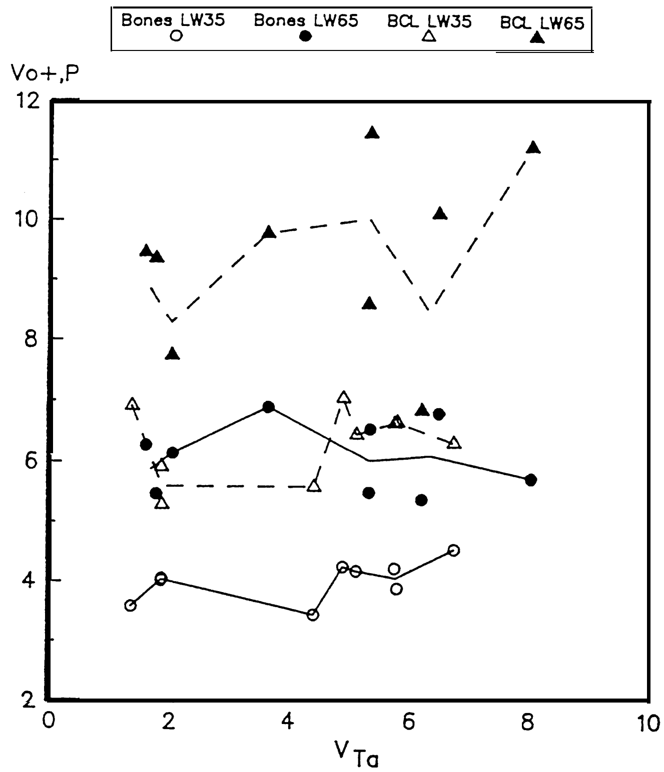

Fig. 7. P accretion (Vo+) estimated by bone radioactivity and the BCL-formulation in relation to live weight (LW) and total mineral absorption (VTa)

The daily accretion of Ca and P estimated by the bone radioactivity and the BCL-formulation are plotted as a function of the total mineral absorption (VTa) in Fig. 6 and 7 for Ca and P, respectively.

It can be seen that regardless of LW and mineral absorption both methods yielded similar estimates ($r=0.89$ for Ca, $r=0.85$ for P) although at different levels.

The accretion rate within LW was rather constant and independent of total mineral absorption from the gut. Average values of each LW are shown in Table 2.

Table 2. The accretion rate (g/day) of Ca and P estimated by the radioactivity in bones and the BCL-formulation

	Live weight				Overall	
	35		65			
	mean	CV	mean	CV	mean	CV
<u>BCL-formulation</u>						
Ca	6.1	14	10.7	5	8.4	29
P	6.3	10	9.4	6	7.8	25
<u>Bones</u>						
Ca	5.9	11	8.6	7	7.2	21
P	4.0	8	6.1	10	5.0	23

The accretion rate of bones, as estimated by their radioactivity content, was significantly higher for the 65 kg pigs than the smaller pigs, a natural consequence of the larger bone mass of the larger pigs. Values obtained by the BCL-formulation, although similar, were higher than those of the former method.

Accretion rates of P obtained by the BCL-formulation are by definition rates of accretion of the whole body. Therefore, the substantially higher BCL-values of P (Fig. 7 and Table 2) are most likely a reflection of the accretion rate of bones and soft tissues.

Mineral removal from bones (Vo-) was calculated as the difference between bone accretion measured by bone radioactivity and mineral balance. The results are shown in Fig. 8 and 9 for Ca and P, respectively.

Mineral resorption from bones was negatively correlated to intestinal mineral input as evidenced by the equations below:

Ca:	LW35, Vo- = 5.4 - 0.7 VTa	$r^2 = 0.86$	eq.no. 9
	LW65, Vo- = 9.1 - 0.9 VTa	$r^2 = 0.87$	eq.no. 10
P:	LW35, Vo- = 3.7 - 0.6 VTa	$r^2 = 0.94$	eq.no. 11
	LW65, Vo- = 5.9 - 0.7 VTa	$r^2 = 0.89$	eq.no. 12

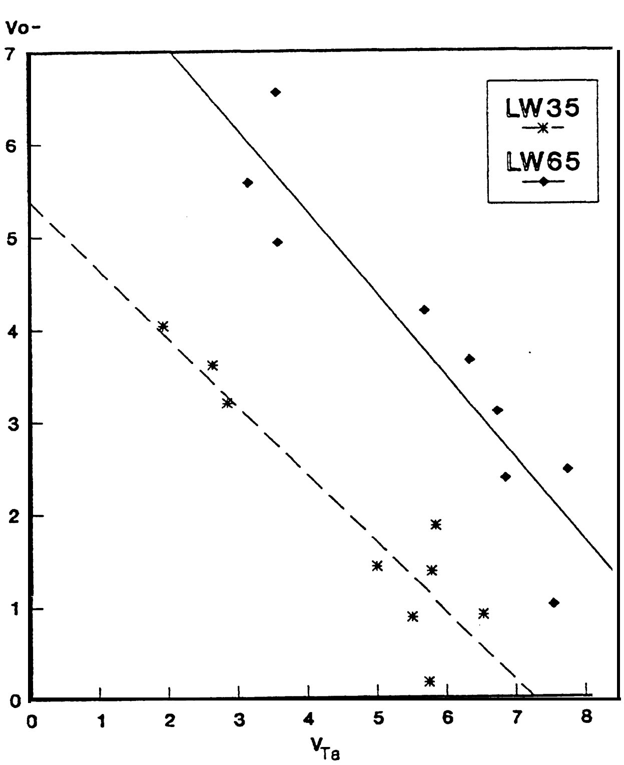

Fig. 8. Resorption of Ca (Vo-) estimated by bone radio-activity in relation to live weight (LW) and total mineral absorption (VTa)

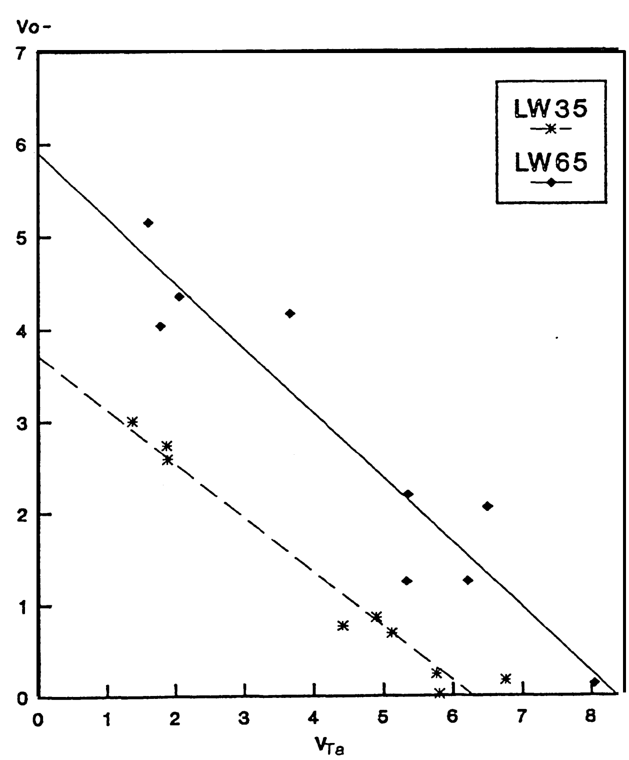

Fig. 9. Resorption of P (Vo-) estimated by bone radio-activity in relation to live weight (LW) and total mineral absorption (VTa)

The above equations (9-12) show that a linear negative relationship with total intestinal input, explained about 90% of the variation of bone resorption of Ca and I? Physiologically, it would have been more meaningful if resorption would have approached zero asymptotically with increasing VTa, as the notion of zero resorption in growing pigs it is not in keeping with the present knowledge of bone development (Dellmann and Brown 1987).

However, the equation with the natural logarithm of VTa did not fit the data as well as the linear equations, possibly because of the relatively large experimental error connected with each single observation, specially when it is considered that the values were generated as differences.

But, whatever the appropriate function, the results clearly show that the ultimate regulatory function of bone is exerted through resorption. Sammon *et al.* (1970) in experiments with growing rats found a similar pattern of Ca metabolism in bones: Ca accretion was independent of mineral intake, while resorption decreased with increasing intake.

Furthermore, the data of Sammon et *al.* (1970) showed a remarkable similarity between the asymptote value of Ca-balance in relation to intake and the average accretion rate.

Analogically, the Ca-balance data of the present study was fitted to an equation of the form:

$$B_a = B_o\,(1-e^{-b*VI}) \qquad \text{eq.no. 13}$$

Where Bo represents the plateau value to which Ca-balance approaches asymptotically at the rate b, with increasing Ca intake (VI).

In Table 3 are shown the Bo values obtained for each LW by eq. 13, the corresponding average values of accretion (Table 2) and the intercept of the equations (9 and 10) fitted to the resorption data, which might be considered as maximum values of resorption.

Table 3. The asymptote values of Ca-balance (Bo), average values of accretion (Vo+) and the intercept (a) of calcium resorption equations.

	Balance	Vo+	Vo-
LW	Bo (Eq. 13)		a (Eq. 9 & 10)
35	6.6	5.9	5.4
65	8.9	8.6	9.1

It can be seen (Table 3) that the maximum Ca retention capacity (Bo), Ca accretion (Vo+) and maximum Ca resorption (a) were also very similar. Results from experiments with normal and parathyroidectomized rats showed (Sammon *et al.* 1970) that parathyroid hormone (PTH) acts primarily on the resorptive processes of bones. Furthermore, Parfitt (1976) proposed that PTH is the principal agent which determine the “Calciostat” directed towards the maintenance of a constant Ca level in plasma.

Consequently, the similarity between Bo, Vo+ and a (Table 3) might be interpreted as the result of the integrated effect of this regulatory system, which very likely is orchestrated by PTH.

Assuming that the similarity between Bo and Vo+ has general validity, and here it must be noted that these estimates were obtained completely independent of each other, in contrast to resorption estimates, then it is possible to acquire knowledge of bone accretion by routine balance procedures, which apart from being less laborious and less complicated than kinetic studies with radioisotopes, make it possible to involve larger numbers of experimental animals under much less strained experimental conditions.

From a nutritional point of view, however, the implication arised by these findings can be illustrated by the calculated parameters of Ca and P metabolism (Table 4) for a pig of 40 kg LW with a daily intake of Ca and P corresponding to either 100% (normal) or 66% (medium) of the Danish standard (Just et al. 1985).

Table 4. Calculated parameters of Ca and P metabolism for a 40 kg LW pig consuming normal*) or medium[1)] amounts of Ca and P (g / d)

	Ca		P	
	Normal VI	Medium VI	Normal VI	Medium VI
Daily intake (VI)	14.3	9.5	11.7	7.5
Net absorption (Va)	5.6	4.7	5.7	4.3
Balance (Ba)	5.6	4.6	4.0	3.1
Accretion (Vo+)	5.8	5.7	4.2	4.2
Resorption (Vo-)	0.2	1.1	0.2	1.1

1) Normal and medium VI correspond to 100% and 66% of the Danish standards (Just *et al.* 1985)

It is clear from the above exercise that the utilization of mineral in excess of medium intake is very poor as only 21% (5.6 - 4.6)/(14.3 - 9.5) of Ca and 29% of P is retained when the intake is increased from medium to normal.

Despite that the Danish standards are among the highest in the world (Just 1985), the incidence of bone abnormalities is rather high, especially in animals with high lean gain (Jørgensen 1982).

The poor utilization of the marginal mineral ingested, together with the persistency of bone abnormalities suggests that the animals setting for bone growth and development do not fit the setting for lean growth.

Furthermore, the similarity of the asymptote values of retention with the level of bone accretion is indicative of a fixed capacity for mineral accretion. As a consequence, the increased net retention in response to increased mineral intake will contribute to bone growth, but the concomitant reduction of bone resorption may impair bone remodelling during growth and thereby the normal development of bone.

Although the results of this investigation does not provide a fully substantiated explanation of mineral metabolism, they do show that achievement of optimum mineral supply to high performing growing pigs, is not a question of increased mineral intake, but is rather a question of manipulating the factors governing maximal bone accretion or a question of finding an intake level which results in resorption levels adequate for bone development and bone growth. At any rate, future research should be directed in the first place to the identification and formulation of a criteria to determine adequate bone growth and development in relation to production.

Since mineral utilization was found to be a function of mineral intake, the degree of utilization being determined by the physiology of the individual animal, then it is obvious that utilization level has to be included in the assessment of the animals' requirements. Evaluation of mineral sources can then be confined to the assessment of the mineral solubility in the particular source, that is, the amount of mineral which is absorbable by normal gastrointestinal processes.

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