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The effects of dietary calcium and phosphorus on the retention and excretion of lead in rats

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1. Rats were given diets containing different amounts of calcium, phosphorus and lead.
2. Pb retention was greatly increased when the diets contained less Ca or P than the minimum estimated requirement of the rat.
3. The release of Pb already incorporated into the skeleton was inhibited by diets low in Ca but was not affected by diets low in P.
4. The retention of Pb given intraperitoneally was not affected by dietary Ca or P.
5. It can be concluded that dietary Ca and P influence the absorption of Pb by the gut and dietary Ca influences the metabolism of Pb in the bone.
6. There were changes in the distribution of Pb among the tissues due to changes of dietary Ca.
7. At low body concentrations, Pb probably affected skeletal growth.

The absorption and retention of lead is influenced by a number of common dietary constituents. Early reports agreed that an increase in dietary calcium resulted in a decreased retention of Pb and some workers found that an increase in dietary phosphorus resulted in decreased Pb retention (Shields & Mitchell, 1940; Sobel, Yuska, Peters & Kramer, 1940) but it has been reported also that P had no effect (Lederer & Bing, 1940). Vitamin D supplementation increased Pb uptake by bone (Sobel *et al.* 1940). Lederer & Bing (1940) found that the retention of Pb given intraperitoneally was the same in rats given a diet adequate in both Ca and P as in rats given a diet low in both minerals. They concluded that the interaction of Ca with Pb takes place in the gut.

After an interval of several decades interest in the possible interaction of nutrients with Pb has been revived and in the last few years it has been reported that the retention of orally ingested Pb by the rat is increased by reducing the dietary Ca content (Six & Goyer, 1970; Mahaffey, Goyer & Haseman, 1973) and decreased by a simultaneous increase in dietary Ca and P, and by the addition of alginate to the diet (Kostial, Šimonović & Pišonić, 1971). The retention of Pb was increased in rats by iron deficiency (Six & Goyer, 1972) and decreased in foals by very high dietary zinc contents (Willoughby, MacDonald, McSherry & Brown, 1972). Diets low in Ca and P had no effect on tissue Pb contents in the horse, except for the liver, in which the Pb content was increased (Willoughby, Thirapatsakun & McSherry, 1972). An increase in dietary P reduced the plasma and bone Pb contents of lambs given Pb in the diet (Morrison, Quarterman & Humphries, 1974).

All these reports have been concerned with the effects of dietary composition on Pb uptake. One report (Sobel & Burger, 1955) discussed the effects of various dietary Ca and P contents on the loss of Pb from rats which had previously been given Pb in

Table 1. *Diet and treatment groups, supplements, and calcium, phosphorus and lead contents of diets (by analysis) given to rats*

Diet and treatment group	Supplements to basal diet*	Content of diet (/kg)		
		Ca (g)	P (g)	Pb (mg)
Control	Ca+P	6·5	7·5	20
Pb-control	Ca+P+Pb	6·4	7·3	202
Low-Ca	P+Pb	2·0	7·3	214
Low-P	Ca+Pb	6·1	3·1	240
Low-Ca–low-P	Pb	1·6	3·0	240

* Contained (/kg): ground wheat 290 g, ground barley 600 g, blood meal 100 g, yeast 10 g, retinol 1·2 mg, cholecalciferol 20 μg; about 1·8 g Ca, 3·0 g P, 20 mg Pb.

their diet. It was found that a low dietary Ca:P ratio reduced the rate of loss of Pb from bone as did vitamin D supplements.

These dietary effects on Pb retention are very large and could have an important influence on the extent to which animals and human subjects are affected by exposure to a given level of dietary or environmental Pb. In the present experiments we have studied the effect of reduced dietary Ca and P contents, separately and together, on the uptake of Pb from the diet, and its subsequent release from the body.

EXPERIMENTAL

Diets and treatments

A basal diet was prepared which contained (/kg): ground wheat 290 g, ground barley 600 g, blood meal 100 g, yeast 10 g, retinol 1·2 mg, cholecalciferol 20 μg. This diet contained (/kg) about 1·8 g Ca, 3·0 g P, 20 mg Pb, and was supplemented, as required, with calcium carbonate, disodium phosphate and lead acetate to give the experimental diets, each of which was pelleted. The amount of food supplied in the hopper of each cage was recorded and no correction was made for wastage.

In each experiment there were five treatments. Details of diets and treatment groups, the supplements for the various treatments and the contents of Ca, P and Pb in each diet (by analysis) are given in Table 1.

Experimental procedure

Expt 1. Eighty female black-hooded Lister rats weighing 185–200 g were randomly divided into five groups of sixteen rats each. Each group received one of the five supplemented diets (Table 1) and tap-water *ad lib.* for 8 weeks. They were kept eight rats/cage and the total food intake was recorded for the group of rats in each cage. At the end of the 8-week period eight rats from each treatment group (four chosen at random from each cage) were killed for analysis. The remaining rats were then given, for a further 8-week period, diets identical to those they received during the first period except that the Pb supplements were omitted. They were then killed for analysis.

Expt 2. In this experiment the effects of diet on Pb retention when the Pb was

given in the diet were compared with the effects when Pb was given intraperitoneally. Fifty male black-hooded Lister rats weighing about 60 g were used. Five groups of five rats were each given one of the five diets described in Table 1 for 7 weeks and then killed for analysis.

The other twenty-five rats were given Pb (as a solution containing 1 mg Pb as lead acetate/ml water) intraperitoneally. The rats were divided into five groups each of five rats. Three groups were each given one of the following diets: Pb-control, low-Ca, low-Ca–low-P (see Table 1), and two groups were given the low-P diet; Pb was omitted from all diets. It was necessary to use two low-P groups because, although rats in the other groups had similar rates of growth, those given the low-P diet grew very slowly; different amounts of Pb were given to these two groups. Rats in the Pb-control, low-Ca, low-Ca–low-P groups, and one low-P group were given the same quantity of Pb (100 μg/rat per d for 15 d during the final 3 weeks of the experiment), and rats in the second low-P group were given an amount of Pb which was related to their body-weight (BW) so that they received the same amount of Pb/g BW as the rats in the groups which grew well, a total of about 525 μg Pb/rat. The experiment involving the low-P rats given Pb according to BW was done separately and subsequently.

Analytical methods

Rats were anaesthetized with sodium pentobarbitone and blood was taken from the heart. The livers and kidneys were removed and weighed. The gastrointestinal tracts were discarded. The remainder of the body (carcase) was dried, ashed at 490° and dissolved in 5 M-nitric acid; the samples of livers and kidneys were treated similarly. Zn, copper, manganese, Fe and Pb were estimated in ashed samples by atomic absorption spectrophotometry. Pb was first extracted into methyl isobutyl ketone as the Pb-ammonium tetramethylene dithiocarbamate complex (Kubasik, Volosin & Murray, 1972). Ca and P in ash and plasma samples were determined by automated procedures (Roach, 1965; Young, 1966; Gitelman, 1967). Erythrocytes were haemolysed using aqueous Triton X-100 before extraction and analysis of Pb as described previously. Haemoglobin was determined by the method of Nicholas (1951) and δ-aminolaevulinate dehydratase (*EC* 4.2.1.24) (ALAD) in blood by the method of Burch & Siegel (1971). One unit of activity is defined as an increase in extinction at 555 nm (with a 10 mm light path) of 0·100/ml erythrocytes per h at 38°.

Statistical analysis

Most of the data obtained in these experiments were not normally distributed and the significance of the differences between groups of data was assessed by a non-parametric method, the Mann-Whitney U test, which essentially tests the extent of overlap of two sets of data. It was used for three comparisons as follows: (*a*) to assess the significance of the effects of adding Pb to the control diet; (*b*) to examine the effect of reducing the Ca level. This had to be done in two parts, comparing first the Pb-control with the low-Ca diet, then comparing the low-P with the low-Ca–low-P diet. Providing the two differences were in the same direction, a combined measure

of significance was obtained by Fisher's technique of combining values of χ^2 corresponding to each of the individual levels of significance; (*c*) the effect of reducing the P level was tested in the same way as that for Ca.

RESULTS

Expt 1. *Effects of dietary Ca and P during Pb ingestion* (*period* 1)

The rats in all groups were given about 2200 g food/rat during the 8-week experimental period, so that the animals in groups 2–5 ingested a maximum of 440 mg Pb. Growth was not affected by the inclusion of Pb but was reduced by low levels of dietary Ca (Tables 2 and 3).

The retention of Pb by the carcase was greatly influenced by diet (Tables 2 and 3); both low-Ca and low-P diets resulted in a retention several times greater than that with the control diet. When both Ca and P were low the effects were approximately additive. (Values for the retention of Pb from the food are minimum values because the values for food intake are maximum values.) Table 2 gives values for the concentration of Pb in the kidneys, liver and erythrocytes of the same rats. Low dietary Ca resulted in a large increase in Pb concentration in all three tissues while low dietary P had an effect only on the liver. Carcase Ca and P (g/kg BW) were not significantly affected by the diet. Mean body Ca (g) was, however, reduced by the addition of Pb to the control diet (control group $3{\cdot}50 \pm 0{\cdot}19$, Pb-control group $2{\cdot}92 \pm 0{\cdot}07$). The contents of Fe, Zn, Cu and Mn in the carcase and in the livers and kidneys were not affected by diet (results not given).

Plasma Ca concentration at the end of period 1 was reduced in the low-Ca group and tended to be lower in the two groups receiving low dietary P. Plasma P concentrations did not change significantly. Haemoglobin levels were not affected but the packed cell volume was increased in the low-Ca and low-P groups. Erythrocyte ALAD activity varied greatly with treatment and was inversely related to erythrocyte Pb concentration according to the regression equation:

$$\text{ALAD (U/ml)} = -15{\cdot}3 \log \text{erythrocyte Pb } (\mu\text{g/ml}) - 0{\cdot}8 \text{ (residual SD } 2{\cdot}32).$$

Kidney and liver Ca concentrations were not affected by dietary Pb or Ca but kidney Ca was greatly decreased and liver Ca was increased by low dietary P (Table 2).

Effects of Ca and P when no Pb was given in the diet (*period* 2)

The rats in every group gained weight during this period, except those in the low-Ca–low-P group (Tables 2 and 3).

The most significant finding at the end of this second period was that the mean total Pb content in the carcases of rats in the low-Ca group was the same as that at the end of the first period, while that in the Pb-control and low-P groups had decreased by half and that in the low-Ca–low-P group had decreased by one-third (Table 2). The Pb contents of the kidneys, livers and erythrocytes had decreased greatly from the levels at the end of the first period (Tables 2 and 3).

Carcase P was significantly reduced by the addition of Pb to the diet (Tables 2

Table 2. *Expt* 1. *The effects of dietary calcium, phosphorus and lead on body-weight, Pb retention, haematological values and Ca, P and Pb contents of the carcase, plasma, liver and kidneys of female rats, weighing* 185–200 *g, given these diets for two* 8*-week periods, at the end of period* 1 *in which dietary Pb supplements were given and at the end of period* 2 *in which Pb supplements were omitted*

(Mean values with their standard errors for eight rats/treatment)

		Diet*									
		Control		Pb-control		Low-Ca		Low-P		Low-Ca–low-P	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body-wt (g)	Period 1	262	8	255	3	240	5	256	2	238	8
	Period 2	291	8	281	5	256	9	270	8	231	7
Carcase Pb (μg)	Period 1	39	3	598	61	2311	126	1600	301	4375	530
	Period 2	32	3	286	16	2244	166	762	169	2809	89
Minimum Pb retention (mg/g ingested)	Period 1	—		1·4		5·2		3·6		10·0	
Erythrocyte Pb (μg/l)	Period 1	98	10	325	32	814	50	379	12	833	37
	Period 2	55	7	146	8	343	35	129	4	369	37
Kidney Pb (μg/g)	Period 1	2·1	0·4	7·2	0·7	38·2	2·8	9·2	0·7	44·2	1·6
	Period 2	1·0	0·1	4·6	1·4	28·3	1·2	2·1	0·4	14·6	0·9
Liver Pb (μg/g)	Period 1	0·24	0·03	0·75	0·09	4·60	0·15	1·58	0·10	3·82	0·19
	Period 2	0·25	0·02	0·24	0·02	0·76	0·03	0·35	0·02	0·97	0·04
Erythrocyte ALAD (U/ml erythrocytes)	Period 1	17·6	0·7	8·4	0·7	0·6	0·5	5·6	0·4	2·6	0·3
	Period 2	18·5	0·9	14·3	0·7	3·1	0·2	8·8	0·4	4·6	0·5
Packed cell volume	Period 1	0·381	0·007	0·380	0·006	0·413	0·005	0·404	0·004	0·389	0·004
	Period 2	0·389	0·006	0·401	0·006	0·420	0·004	0·408	0·004	0·317	0·004
Haemoglobin (g/l)	Period 1	130	4	128	3	130	1	131	2	126	2
	Period 2	126	2	132	1	134	1	130	1	131	1
Carcase Ca (g/kg body-wt)	Period 1	13·6	1·0	11·4	0·3	11·9	0·6	10·6	0·4	10·9	0·6
	Period 2	13·8	0·5	12·5	0·6	11·6	0·3	10·8	1·0	13·0	0·5
Carcase P (g/kg body-wt)	Period 1	7·2	0·5	6·6	0·2	6·6	0·2	6·2	0·2	6·4	0·3
	Period 2	8·1	0·3	7·3	0·2	6·8	0·2	6·8	1·1	7·5	0·3
Plasma Ca (mmol/l)	Period 1	2·54	0·07	2·57	0·02	2·09	0·13	2·42	0·06	2·40	0·06
	Period 2	2·67	0·05	2·61	0·03	2·57	0·05	2·65	0·03	2·54	0·03
Plasma P (mmol/l)	Period 1	1·69	0·03	1·59	0·05	1·87	0·11	1·72	0·10	1·71	0·08
	Period 2	1·73	0·06	1·78	0·10	1·54	0·10	2·09	0·11	1·94	0·08
Kidney Ca (μg/g)	Period 1	100	17	110	11	88	9	6	1	6	1
Liver Ca (μg/g)	Period 1	1·2	0·1	1·0	0·1	1·2	0·2	1·8	0·1	2·5	0·3

ALAD, δ-aminolaevulinate dehydratase (*EC* 4.2.1.24) (for definition of units, see p. 353).

* For details, see Table 1.

Table 3. *Expt* 1. *The results of a non-parametric analysis (Mann-Whitney U test) of the data given in Table* 2 *which indicate the significance of the dietary treatments on the variables which were measured*

Variable		Effect of adding lead to control diet	Effect of reducing calcium content of diet	Effect of reducing phosphorus content of diet
Body-wt (g)	Period 1	NS	*dec	NS
	Period 2	NS	**dec	NS
Carcase Pb (μg)	Period 1	**inc	**inc	**inc
	Period 2	**inc	**inc	*inc
Erythrocyte Pb (μg/l)	Period 1	**inc	**inc	NS
	Period 2	**inc	**inc	NS
Kidney Pb (μg/g)	Period 1	**inc	**inc	NS
	Period 2	**inc	**inc	NS
Liver Pb (μg/g)	Period 1	**inc	**inc	**inc (*dec with low-Ca)
	Period 2	NS	**inc	**inc
Erythrocyte ALAD (U/ml erythrocytes)	Period 1	**dec	**dec	**dec (**inc with low-Ca)
	Period 2	**dec	**dec	**dec (NS with low-Ca)
Packed cell volume	Period 1	NS	**inc	*inc (**dec with low-Ca)
	Period 2	NS	*inc	NS
Haemoglobin (g/l)	Period 1	NS	NS	NS
	Period 2	**inc	NS	NS
Carcase Ca (g/kg body-wt)	Period 1	NS	NS	NS
	Period 2	NS	NS	NS
Carcase P (g/kg body-wt)	Period 1	NS	NS	NS
	Period 2	*dec	NS	NS
Plasma Ca (mmol/l)	Period 1	NS	**dec (NS with low-P)	NS
	Period 2	NS	NS	NS
Plasma P (mmol/l)	Period 1	NS	NS	NS
	Period 2	NS	NS	**inc
Kidney Ca (μg/g)	Period 1	NS	NS	**dec
Liver Ca (μg/g)	Period 1	NS	NS	**inc

ALAD, δ-aminolaevulinate dehydratase (*EC* 4.2.1.24) (for definition of units, see p. 353); NS, not significant; inc, increase; dec, decrease; low-P, low-Ca, groups receiving diets with no supplementary P and Ca respectively (for details, see Table 1).

* $P < 0.05$, ** $P < 0.01$.

and 3). The difference in the carcase Ca content (g/kg) (Table 2) between the Pb-control group and the control rats was not significant although the total amounts of Ca (g) at the end of period 2 were significantly different (3.52 ± 0.10 and 4.01 ± 0.12 respectively).

Values obtained at the end of period 2 indicated that plasma Ca was unaffected by diet but plasma P was increased by the low-P diet. The addition of Pb increased haemoglobin concentration and the packed cell volume was increased in the low-Ca group.

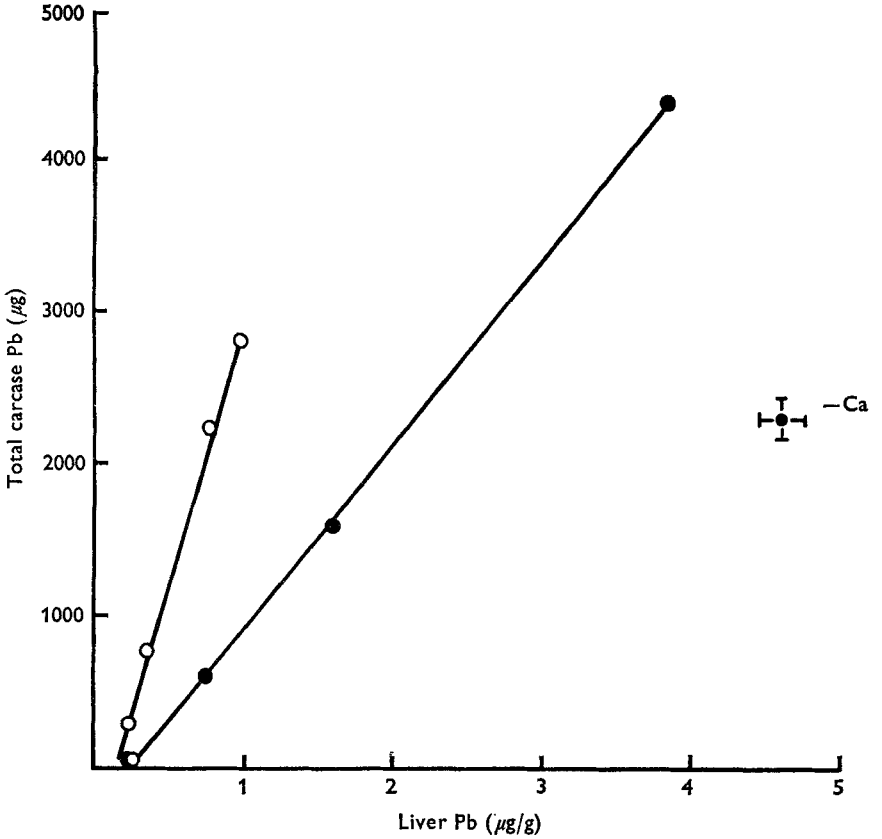

Fig. 1. Expt 1. The relationship between total carcase lead (μg) and liver Pb concentration (μg/g) for five groups of female rats, weighing 185–200 g, given diets with different calcium, phosphorus and Pb contents. The points represent mean values for eight rats/group killed at the end of period 1 (●) and at the end of period 2 (○) of the experiment (for details of diets and treatments, see p. 353 and Table 1). The point (–Ca) which deviates from the lines represents the mean value for rats which received the low-Ca diet and were killed at the end of period 1; the standard errors for this point are represented by the bars.

Distribution of Pb

There was a linear relationship between the contents of Pb in any two tissues (including the carcase) at both stages of the experiment (periods 1 and 2). Deviations from this general relationship occurred in some of the low-Ca groups, and were such that in these groups the soft tissues had proportionally more Pb in relation to the carcase than the tissues of rats in other groups. One of these relationships is shown for the liver in Fig. 1.

Expt 2

In this experiment the rats were younger than those used in Expt 1 and they were males. They grew at a faster rate and, by contrast with Expt 1, growth was severely reduced with the low-P diet (Tables 4 and 5). The low-Ca–low-P diet in which the Ca:P ratio was similar to that in the control diets allowed a rate of growth not significantly different from the control and low-Ca diets.

Table 4. *Expt 2. The effects of dietary calcium and phosphorus, and of dietary (O) or intraperitoneal (IP)* lead on the body-weight, Ca and P contents of the carcase and plasma, and the Pb contents of the carcase and erythrocytes of male rats, weighing 60 g, given these diets for 7 weeks*

(Mean values with their standard errors for five rats/treatment)

		Diet†											
		Control		Pb-control		Low-Ca		Low-P		Low-P‡		Low-Ca–low-P	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body-wt (g)	O	309	6	298	11	287	14	190	13	—		265	11
	IP	287	7	—		296	7	137	13	117	5	296	2
Carcase Pb (μg)	O	58	7	654	67	1965	67	1454	98	—		3758	73
	IP	1082	77	—		994	50	1020	27	242	9	1056	53
Erythrocyte Pb (μg/l)	O	71	17	579	57	1031	67	1063	46	—		1595	57
	IP	762	102	—		707	41	1337	175	686	86	822	29
Carcase Ca (g/kg body-wt)	O	99	5	99	3	91	2	102	5	—		87	9
	IP	104	3	—		87	4	101	4	91	3	84	3
Carcase P (g/kg body-wt)	O	66	2	68	2	63	1	64	3	—		54	4
	IP	68	1	—		59	2	65	2	56	1	61	2
Plasma Ca (mmol/l)	O	2·72	0·02	2·71	0·02	2·74	0·02	3·18	0·06	—		2·73	0·04
	IP	2·72	0·04	—		2·81	0·06	3·10	0·07	—		2·78	0·05
Plasma P (mmol/l)	O	2·90	0·22	2·59	0·20	2·45	0·07	1·70	0·08	—		2·61	0·12
	IP	3·16	0·09	—		3·01	0·29	1·62	0·03	—		2·96	0·17

* Each rat in each group (except low-P‡) received 1500 μg intraperitoneally (100 μg/rat per d) for 15 d during the final 3 weeks of the experiment.
† For details, see Table 1.
‡ Rats in this group were given the same amount of IP Pb/kg body-wt as rats in the control group.

Table 5. *Expt 2. The results of a non-parametric analysis (Mann-Whitney U test) of the data given in Table 4 which indicate the significance of the dietary treatments on the variables which were measured*

Variable		Effect of adding lead to control diet	Effect of reducing calcium content of diet	Effect of reducing phosphorus content of diet
Body-wt (g)	O	NS	**inc (NS with high-P)	**dec (NS with low-Ca)
	IP	NS	**inc (NS with high-P)	**dec (NS with low-Ca)
Carcase Pb (μg)	O	**inc	**inc	**inc
	IP	**inc	NS	NS
Erythrocyte Pb (μg/l)	O	**inc	**inc	**inc
	IP	**inc	**dec (NS with high-P)	**inc (NS with low-Ca)
Carcase Ca (g/kg body-wt)	O	NS	NS	NS
	IP	NS	**dec	NS
Carcase P (g/kg body-wt)	O	NS	*dec	NS
	IP	NS	*dec	NS
Plasma Ca (mmol/l)	O	NS	**dec (NS with high-P)	**inc (NS with low-Ca)
	IP	NS	**dec (NS with high-P)	**inc (NS with low-Ca)
Plasma P (mmol/l)	O	NS	**inc (NS with high-Ca)	**dec (NS with low-Ca)
	IP	NS	**inc (NS with high-Ca)	**dec (NS with low-Ca)

O, Pb added to diet; IP, intraperitoneally administered Pb; NS, not significant; inc, increase; dec, decrease; high-Ca, high-P, groups receiving diets with supplementary Ca and P respectively; low-Ca, groups receiving diets with no supplementary Ca.
* $P < 0.05$, ** $P < 0.01$.

Effects of Ca and P on dietary Pb retention

The total amounts of Pb in the carcases of rats given Pb in the diet were affected by dietary Ca and P in a similar manner to those in Expt 1 (Tables 4 and 5). However, the carcase concentration of Pb (μg/g) in the low-P rats was greater than that in the low-Ca rats, in contrast with results obtained in Expt 1. It is apparent that the erythrocyte Pb concentrations reflect the carcase concentration of Pb (μg/g) more closely than the absolute amounts of Pb (μg) present.

Plasma Ca was increased and plasma P was decreased by the low-P diet. The low-Ca diet decreased plasma Ca and increased plasma P. In the rats treated intraperitoneally, carcase Ca was decreased by the low-Ca diet (Tables 4 and 5). Packed cell volumes were not affected by any treatment (results not given).

Effects of Ca and P on retention of intraperitoneally-administered Pb

The retention of Pb given intraperitoneally was not affected by dietary Ca or P concentrations. About two-thirds of the Pb given intraperitoneally was retained in the carcases of all groups irrespective of diet (Table 4), except in the low-P group given Pb according to BW; this group received about one-third of the amount of Pb given to the other groups, of which less than half was retained. In the low-P group the plasma Ca was increased and the plasma P decreased. There was a decrease in carcase P in the low-Ca group. Erythrocyte Pb concentrations varied with carcase Pb concentration (μg/g) rather than with total Pb content.

Distribution of Pb

In this experiment in which the rats were younger, more rapidly growing than those in Expt 1, and male rather than female, a given concentration of carcase Pb was associated with a higher concentration of Pb in the erythrocytes than in Expt 1 (Tables 2 and 4).

DISCUSSION

The results of this work indicate clearly that a reduction of dietary Ca and P to levels below requirement caused a very large increase in the retention of Pb given with the food. The low concentrations of Ca and P used were about 33 and 70% respectively of the recommended allowances for growing rats ((US) National Research Council, 1972). Compared with the effects on Pb uptake these diets produced only small changes in growth or Ca and P levels in plasma and carcase compared with rats given control diets containing more than adequate amounts of Ca and P, except in the rapidly growing male rats of Expt 2, which were greatly affected by the two diets low in P.

The increased uptake of Pb was related to the actual Ca and P contents of the diet and not to the ratio of these elements, or the plasma or carcase Ca or P, or the rate of growth of the rat. The low level of dietary Ca was a much smaller proportion of the recommended allowance than was the low level of dietary P. Nevertheless in Expt 2 with young male rats the low-Ca and the low-P diets caused similar increases in Pb retention.

Six & Goyer (1970) who used diets containing 1·0 g Ca/kg and supplemented the water with 200 μg Pb/ml, obtained higher concentrations of Pb in blood and kidney than those obtained in this work. The higher Pb uptake in their work was probably associated with the lower dietary Ca content. They also found a retention of 30–70% of dietary Pb which is much higher than that reported by Blaxter (1950) for sheep (1·3%) and rabbits (1·0%) and by Kehoe (1961) for humans (about 10%), who all reported higher retentions with an adequate diet than those found in this work with rats.

There was no effect of diet on the retention of Pb given intraperitoneally and it is probable therefore that dietary Ca and P influence Pb absorption from the gut.

Vitamin D supplementation and low dietary Ca and P are conditions which increase Pb absorption and which also increase the concentration of Ca-binding protein (CaBP) in the intestinal mucosa (Wasserman & Corradino, 1973). The results of experiments (Quaterman & Morrison, unpublished results) done in this laboratory have indicated that the increased Pb absorption found after vitamin D dosing of vitamin D-depleted rats was inhibited by the intraperitoneal administration of the protein-synthesis inhibitor cycloheximide. Thus dietary effects on Pb absorption may be accounted for, in part, by changes in the concentration of CaBP, although other sorts of interactions between Pb and Ca and P have been shown to occur in heart mitochondria (Scott, Hwang, Jurkowitz & Brierley, 1971).

In this work the release and excretion of Pb already incorporated into the carcase

was inhibited by diets low in Ca and the extent of this inhibition was reduced when the diet was also low in P. These large changes in carcase Pb cannot be accounted for by changes in soft tissue Pb and must indicate the release of Pb from bone. This is because the Pb content of the soft tissues (mainly muscle) of the carcase is very low by comparison with the Pb content of bone, except possibly at the lowest Pb levels (Schroeder & Tipton, 1968) and the carcase Pb contents in this work may be taken as approximately equal to the contents of Pb in the skeleton.

Evidence that these effects of diet on Pb retention in the skeleton may be independent of skeletal formation and resorption has been obtained in work with lactating rats using similar diets which produced very severe resorption. Thus, during a period of Pb dosing, total body Ca was reduced from about 2·3 to 1·9 g by low-Ca or low-P diets but Pb retention increased from 350 to 1200 μg. In the subsequent gestation when no Pb was given, carcase Ca increased to 2·2 g with these diets but no Pb was lost. In the following lactation period, again with no Pb feeding, there was severe loss of carcase Ca to about 1·2 g but no significant amounts of Pb were excreted (Quarterman, Morrison & Carey, 1974). The extent of Pb retention by the skeleton may be further affected by the age or sex, or stage and rate of growth of the animal; for example the relationship between carcase Pb and erythrocyte Pb was different in the two experiments reported here.

The amounts of Pb given to the rats were small and the highest blood concentrations produced were below those regarded as the upper limit of the normal range for human subjects (British Medical Journal, 1968). The amounts retained by the Pb-control group were however sufficient to produce a decrease in carcase P compared with the control group in Expt 1. According to Hsu, Krook, Shively, Duncan & Pond (1973) bone mineralization is affected by Pb by an alteration of osteocyte activity resulting, in the early stages, in excessive mineral deposition at the metaphyses and later in osteoclasis. It seems possible that the osteoclastic stage had been reached after 8–16 weeks even with mild exposure to Pb. There was no effect of dietary Pb on carcase Ca or P (g/kg body-weight) in the more rapidly growing male rats.

A large part of the Pb deposited in the kidney and liver is in the form of intranuclear inclusion bodies (Goyer, May, Cates & Krigman, 1970) which have been reported to contain Ca (Carrol, Spinelli & Goyer, 1970) and Fe (Galle & Morel-Marager, 1965) as well as protein and phosphate. In this work, however, there was no increase in Ca or Fe in these tissues as the Pb concentration increased, although kidney Ca was found to be very dependent on dietary P (cf. Laflamme & Jowsey, 1972).

The effects of Pb on ALAD activity are similar to those reported for human subjects (Hernberg, Nikkanen, Mellin & Lilius, 1970; Haeger-Aronsen, Abdulla & Fristedt, 1971) although the activity of the enzyme in rat blood is about one-quarter that in human blood. In Expt 1 packed cell volumes and haemoglobin concentrations were increased above control values in low-Ca and low-P groups. These increases may be the result of a small stimulation of erythropoiesis which has been found in response to mild Pb poisoning (de Bruin, 1971).

The effects of dietary Ca and P reported in this paper, and by other workers, are

very large and the response of man or animals exposed to Pb in food or in the air could vary considerably depending on the nature of the diet. This aspect of Pb metabolism has been largely ignored in clinical and epidemiological studies of Pb poisoning (e.g. WHO, 1972).

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