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TEXAS AGRICULTURAL EXPERIMENT STATION

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College Station, Brazos County, Texas

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DIVISION OF CHEMISTRY

The Relation of Lime and Phosphoric Acid to the Growth and Bone Development of White Rats

AGRICULTURAL AND MECHANICAL COLLEGE OF TEXAS
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In cooperation with U. S. Department of Agriculture.

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The importance of a sufficient quantity of lime, phosphoric acid, and other minerals in the food of man and animals is generally recognized.

The effect of the quantity and of the ratio of lime to phosphoric acid in the feed was studied with rats because the results might have some relation to the needs of man and domestic animals for these minerals.

The ratio of lime to phosphoric acid of 1:1 was found to be favorable for rats when the ration contained 1.0, 1.5, and 3.0 per cent of each mineral.

Small deviations of either lime or phosphoric acid from the favorable ratios or amounts decreased the growth of the rats. The ash in the femurs was in some cases affected by these changes, but not always.

The minimum amounts in the ration which produced normal growth and good bone development were found to be 0.75 per cent of lime and 1.0 per cent of phosphoric acid.

Less than the minimum amounts of lime or phosphoric acid caused decreased growth, produced less than the normal percentage of bone in the rat, and lowered the percentage of ash in the femur.

The excessive percentage of 6.0 per cent of phosphoric acid fed with 1.5 per cent of lime in the ration decreased the growth of the rats considerably, but produced femurs containing practically a normal percentage of ash and developed a large quantity of bone out of proportion to the total weight of the body. On the other hand, 6.0 per cent of lime with 1.5 per cent of phosphoric acid produced normal growth and normal bones.

The ratio of lime to phosphoric acid in the femurs had no relation to the amounts of lime and phosphoric acid fed in the ration.

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BULLETIN NO. 441 DECEMBER, 1931

THE RELATION OF THE LIME AND PHOSPHORIC ACID IN THE FEED TO THE GROWTH AND DEVELOPMENT OF BONES OF WHITE RATS*

By J. K. BLUM

It is a recognized fact that minerals are a very important part of animal foods. Not only the presence and quantity of minerals play a part, but also the ratio of one to the other seems to be a factor in the growth and health of the body.

Of the mineral elements studied, phosphorus and calcium have received the greatest amount of attention. Recent investigations show that the insufficient quantity or an unsuitable ratio of these elements results in retarded growth and the development of diseases related to the skeleton. Osteoporosis, or porous bone, has been traced to an insufficient quantity of either calcium or phosphorus in the diet. Rickets also has been found to be caused by an improper ratio of calcium to phosphorus. While vitamins are important in bone development, they cannot correct deficiencies in mineral.

Poultry, cattle, and other domestic animals as well as man have diseases which are traceable to insufficient quantities or incorrect ratios of calcium to phosphorus in the diet. It is becoming necessary to grow animals at a rapid rate in order to derive a profit from feeding. To do this every food requirement must be supplied in proper quantity.

The study upon which the following discussion is based was undertaken to determine the effect of varying the lime and phosphoric-acid content of the ration upon the growth and the bone development of white rats. While results from experiments on rats cannot be applied directly to other animals, they can be used as a foundation for more extensive study.

A ration was used which was believed to contain all the necessary factors for growth and well-being of white rats, with the exception of lime and phosphoric acid, which were supplied in various amounts. The results of such a study should show the extent to which lime and phosphoric acid affect the health of the animal when all other factors are supplied in proper quantity.

HISTORICAL

McCollum (5) discovered about 1914 that calcium supplied as the lactate when added to a diet made up of parts of the wheat plant in-

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creased the growth of pigs and rats. This was confirmed later by McCollum and co-workers (13) (14) using a different ration and adding calcium carbonate.

Steenbock and associates (23) observed that a ration made up of cereals and gelatin was improved by the addition of one per cent of calcium carbonate. This addition produced better growth in rats than the ration without the mineral. These results were confirmed by Shohl and Karelitz (20).

McCollum and Simmonds (13) found that rats fed a ration containing a high percentage of calcium (3 per cent, calcium carbonate) and a low percentage of phosphorus developed signs of rickets and died early. They implied that the ratio between calcium and phosphorus had more to do with the production of rickets and other similar diseases than the actual quantities of the mineral.

Steenbock (23) concluded that a ration which contained 1.08 per cent of calcium and .254 per cent of phosphorus produced rickets in rats.

McCollum and co-workers (14) produced rickets on rats with a ration made up of purified food substances containing 1.202 per cent of calcium with .217 per cent of phosphorus. This was confirmed by further experimentation (13). They found that a disproportion between calcium and phosphorus with a limited amount of cod liver oil resulted in rickets while an insufficient quantity of calcium and phosphorus produced osteoporosis.

Simmonds (19) claimed that the rats require about .64 gram of calcium per hundred grams of ration.

Nelson, Irwin, and Peet (16) state that 0.5669 gram of calcium and 0.4556 gram of phosphorus in a calcium-to-phosphorus ratio of 1:.80 per hundred grams of ration were sufficient for rats both as to quantity and ratio of calcium to phosphorus.

According to the work of Holmes and Pigott (18), a ratio of calcium to phosphorus of 1.8 to 1.74 produces the best growth in chicks. These same ratios also produced tibiae which contained greater percentages of ash than other ratios. One per cent of cod liver oil was fed to the chicks.

Josef Schneider (17) states that if a normal person receives calcium and phosphorus in the ratio of 1:.444, no vitamin D is necessary.

According to Grieves (4) 0.41 gram of phosphorus per 100 grams of ration is optional for rats. He found that good growth, well-being, and normal osseous tissues were induced in rats with 0.64 gram of calcium and 0.41 gram of phosphorus to 100 grams of rations when vitamin D was added in the form of butter fat or cod liver oil.

The work of Steenbock (23) and associates shows that a ratio of Ca: P within a range of 1:.75 to 1:.50 produced the more favorable quantity of ash in the femurs of rats.

Karelitz and Shohl (10) discovered that rickets could be cured in rats in some cases by correcting the ratio of calcium to phosphorus to 1:.95.

Moritz and Krenz (15) state that if the diet of rabbits contains calcium and phosphorus in the proper ratio, no vitamin D was needed to prevent rickets.

Sherman and Steebeling (18) concluded that either vitamin D, calcium, or phosphorus may prove to be the limiting factor in the development of bone in rats.

About the year 1923 Touverud (25) found that rats which were fed a low calcium diet developed bones which contained smaller percenttages of ash than normal rats.

McCann and Barnett (11) observed that the total ash of rachitic bones is lower than in normal bones, while the ratio of calcium to phosphorus in the ash is not changed.

The work of Steenbock and associates (23) (24), Dutcher and associates (2), (3), McCann and Barnett (11), McCollum and Adams (12), Shohl and co-workers (20) (21), Hess and others (7), Holmes and Pigott (8), all go to show that either a disproportion of calcium to phosphorus in the diet or a lack of sufficient vitamin D results in producing a bone containing less ash than normal. However, there is little variation in the ratio of calcium to phosphorus in the ash of the bone from that of normal, which is approximately 1 : .50.

METHODS AND PROCEDURE

Rats about twenty-three days old and of a weight of thirty-seven to forty grams were placed in separate cages and given a ration made up of the following: 20 parts of casein, 10 parts of dried yeast, 67.5 parts of starch, 1 part of sodium chloride, and 1.5 parts of a salt mixture made up of the following: 68 parts of potassium sulphate, 444 parts of potassium chloride, 96 parts of magnesium carbonate, 62 parts of sodium carbonate, 37 parts of potassium carbonate, 252 parts of citric acid, 24 parts of iron citrate, 0.32 part of manganese sulphate, 1.0 part of sodium fluoride, 0.08 part of potassium iodide, 0.08 part of potassium alum. The percentage of lime (CaO) in this ration was found by analysis to be 0.250 (Ca = 0.178 per cent) and of phosphoric acid (P_2O_5) 0.670 (P = 0.292 per cent). Different levels of lime and phosphoric acid were obtained in the rations by substituting in place of starch, calcium carbonate or disodium phosphate.

Each rat was given plenty of distilled water and three or four drops of cod liver oil (vitamin-tested) per day. Records were kept of the amount of food consumed and the weight gained each week. At the end of eight weeks the rats were killed with chloroform and the femurs dissected out.

The procedure used for determination of the ash in the femurs was based upon that of Dutcher and Honeywell (2) (9). The femurs were cleaned free of flesh and connecting tissues and then weighed. After being dried at a temperature of 70-90° C to constant weight they were weighed again. The samples were then extracted for twenty-four hours

with ninety-five per cent alcohol and then twenty-four hours with ether, after which they were dried and weighed. The ash was prepared from this dried fat-free bone by combustion in an electric furnace, and weighed. The ash was dissolved in 10 cc. of dilute hydrochloric acid (1:1) and the volume made up in 100 cc. An aliquot was taken and the phosphoric acid (P_2O_5) determined by precipitation with ammonium molybdate and titration of the precipitate with potassium hydroxide and nitric acid.

The lime was determined in another aliquot of the solution by precipitating it with ammonium oxalate in the presence of acetic acid. The precipitate after being burned was heated with ammonium sulfate and the calcium was weighed as calcium sulfate, and calculated to calcium oxide.

The Terms Lime and Phosphorus

In making the calculations and ratios the calcium has been expressed as lime (CaO) and the phosphorus as phosphoric acid (P_2O_5). While this is not the custom in animal physiology, the terms lime and phosphoric acid are ordinarily used in agriculture, instead of calcium and phosphorus. The analysis of fertilizer is always, by legal requirement, expressed in terms of phosphoric acid. It was thought that the results might be better interpreted in relation to feeding experiments, where it is customary to use agricultural analyses.

REQUIREMENTS FOR NORMAL GROWTH

The gain in weight of each individual rat is presented in Table 1, along with the percentages of lime and phosphoric acid in the ration. This table shows some variations in the weights of the rats on the same ration. There are several factors that affect the health and growth of rats, such as the inherent vitality and ability of the animal to grow, and this may account for the variation.

In Table 2 there is found the weights of femurs and other data on the bones along with the lime and phosphoric acid in the rations fed. The percentages of the femur and of the femur ash in the rat are given. It is thought that these figures might have some relation to the value of the rations.

In Table 3 some of detailed results in Tables 1 and 2 are averaged and arranged in four groups, according to the weights of the rats. The group containing the largest rats is given first.

The greatest growth of 171 grams was made on a ration containing 6.0 per cent of lime and 1.5 per cent of phosphoric acid with a ratio of 1:.25. Practically the same growth (170 grams) was made by the rats on the ration containing 1.0 per cent of phosphoric acid with a ratio of 1:1, and those on the ration which contained .75 per cent of lime and 1.0 per cent of phosphoric acid. The rats on the other three rations in this group seemed to have normal growth (160-161 grams). The average weight of the rats in this group was 165 grams and the

Rat No.	Lime in ration per cent	Phosphoric acid in ration per cent	Ratio of lime to phosphoric acid in ration	Wt. at beginning grams	Weight in grams at end of each week.							
					1	2	3	4	5	6	7	8
47 M	6.00	1.50	1:.25	40	46	52	70	104	129	158	166	172
48 M	6.00	1.50	1:.25	39	45	54	78	94	110	144	160	170
45 M	4.50	1.50	1:.33	38	56	80	94	110	124	150	158	170
46 F	4.50	1.50	1:.33	39	44	54	74	84	102	126	140	152
29 F	3.00	1.50	1:.50	39	52	68	80	100	106	113	120	135
30 M	3.00	1.50	1:.50	40	54	74	94	118	128	147	166	170
8 F	1.50	.75	1:.50	37	40	68	82	90	104	106	114	125
51 M	1.50	.75	1:.50	37	44	66	80	94	106	108	110	120
52 M	1.50	.75	1:.50	37	56	79	86	102	112	120	124	130
27 F	2.50	1.50	1:.60	40	54	74	82	100	108	112	126	140
28 M	2.50	1.50	1:.60	40	52	76	90	102	110	124	135	150
41 M	1.50	1.00	1:.666	39	54	78	108	130	140	166	170	165
42 F	1.50	1.00	1:.666	37	50	74	98	106	118	130	140	144
25 F	2.00	1.50	1:.75	37	46	46	36	50	72	92	90	110
26 F	2.00	1.50	1:.75	37	47	46	34	44	58	68	78	88
49 M	3.00	3.00	1:1	40	52	78	104	110	130	150	160	166
50 M	3.00	3.00	1:1	40	46	86	90	116	130	144	150	155
15 M	1.50	1.50	1:1	37	44	72	83	106	126	156	172	172
16 M	1.50	1.50	1:1	37	41	66	86	100	118	142	154	165
23 F	1.50	1.50	1:1	39	60	81	94	116	128	138	144	154
24 F	1.50	1.50	1:1	39	62	74	92	100	110	124	138	150
37 F	1.00	1.00	1:1	40	60	88	112	128	136	160	170	174
38 M	1.00	1.00	1:1	40	56	80	100	116	124	144	158	166
5 F	.75	.75	1:1	40	56	82	100	108	128	128	142	158
6 F	.75	.75	1:1	37	40	66	84	98	112	116	126	130
31 F	1.50	2.00	1:1.333	37	50	70	84	86	99	111	118	127
32 F	1.50	2.00	1:1.333	39	50	70	84	104	110	128	136	148
39 F	.75	1.00	1:1.333	40	60	84	112	122	130	150	160	166
40 M	.75	1.00	1:1.333	40	62	82	110	124	136	156	166	174
13 M	1.00	1.50	1:1.50	38	44	52	69	82	100	104	128	142
14 F	1.00	1.50	1:1.50	37	44	72	84	90	112	126	138	150
19 F	.50	.75	1:1.50	40	60	72	88	84	100	112	129	132
20 F	.50	.75	1:1.50	40	44	56	70	82	88	88	96	100
3 F	.50	.75	1:1.50	40	48	68	84	94	104	110	120	120
4 F	.50	.75	!:1.50	36	38	60	81	90	100	108	123	138
33 M	1.50	2.50	1:1.666	37	49	62	70	90	102	110	120	135
34 F	1.50	2.50	1:1.666	40	46	64	76	98	108	122	130	160
35 M	1.50	3.00	1:2	37	46	66	82	100	108	126	132	152
36 F	1.50	3.00	1:2	38	44	62	77	93	102	104	110	140
54 F	1.50	3.00	1:2	40	46	60	82	94	110	120	130	126
55 M	1.50	3.00	1:2	39	48	54	80	90	109	120	142	140
17 F	.25	.67	1:2.68	40	50	50	54	70	74	78	84	81
18 F	.25	.67	1:2.68	40	46	52	52	66	74	76	84	84
11 F	.50	1.50	1:3	37	48	74	91	98	118	120	137	140
12 M	.50	1.50	1:3	38	46	62	83	102	116	118	130	136
1 F	.25	.75	1:3	46	60	78	94	104	114	118	130	142
2 F	.25	.75	1:3	40	44	59	72	88	100	104	116	130
43 F	1.50	6.00	1:4	37	45	58	74	74	74	74	80	70
44 F	1.50	6.00	1:4	37	48	64	80	88	84	90	100	86

Table 2. Weights of femur and femur ash of rats grown with different ratios of lime to phosphoric acid.

Rat Number	Lime in ration per cent	Phosphoric acid in ration per cent	Ratio $CaO:P_2O_5$ in ration	Weight of femurs grams	Dried femurs grams	Extracted femurs grams	Ash of femurs grams	Ash in femurs per cent	Femur in rat per cent	Femur ash in rat per cent
47 M	6.00	1.50	1:.25	1.2728	.8009	.7772	.4545	58.48	.426	.241
48 M	6.00	1.50	1:.25	1.3048	.8047	.7773	.4581	58.93	.442	.251
45 M	4.50	1.50	1:.33	1.0225	.7201	.7053	.4005	56.75	.411	.235
46 F	4.50	1.50	1:.33	.9802	.6496	.6378	.3742	58.66	.427	.246
29 F	3.00	1.50	1:.50	.9701	.6002	.5838	.3482	59.64	.491	.248
30 M	3.00	1.50	1:.50	1.2042	.7148	.6945	.4023	57.93	.420	.236
8 F	1.50	.75	1:.50	.9009	.5464	.4902	.2678	54.63	.455	.223
51 M	1.50	.75	1:.50	.7544	.4938	.4450	.2421	54.40	.448	.220
52 M	1.50	.75	1:.50	.7947	.5316	.4871	.2684	55.10	.428	.216
27 F	2.50	1.50	1:.60	.9891	.5702	.5431	.3184	58.63	.459	.256
28 M	2.50	1.50	1:.60	.9342	.6240	.6128	.3733	60.92	.476	.284
41 M	1.50	1.00	1:.66	1.0717	.7641	.6945	.4513	64.97	.489	.289
42 F	1.50	1.00	1:.66	.9232	.6621	.6423	.4845	75.43	.459	.336
25 F	2.00	1.50	1:.75	.8122	.4904	.4443	.2482	55.86	.445	.225
26 F	2.00	1.50	1:.75	.6648	.3648	.3123	.1482	47.45	.414	.168
49 M	3.00	3.00	1:1	1.1745	.7247	.6643	.3930	59.16	.436	.236
50 M	3.00	3.00	1:1	.8301	.6248	.5691	.3381	59.41	.499	.270
15 M	1.50	1.50	1:1	1.2010	.7220	.6384	.3660	57.33	.419	.212
16 M	1.50	1.50	1:1	1.1488	.6842	.6152	.3575	58.11	.444	.232
23 F	1.50	1.50	1:1	.9948	.6842	.6548	.3882	59.29	.444	.252
24 F	1.50	1.50	1:1	.9500	.6615	.6231	.3721	59.71	.459	.258
37 F	1.00	1.00	1:1	.9801	.7501	.7351	.4470	60.81	.431	.256
38 M	1.00	1.00	1:1	.8515	.6338	.5828	.3380	60.77	.381	.203
5 F	.75	.75	1:1	1.1211	.6972	.6729	.3908	58.08	.440	.240
6 F	.75	.75	1:1	1.0114	.6078	.5501	.3062	55.66	.467	.235
31 F	1.50	2.00	1:1.33	.8402	.5478	.5190	.2842	54.75	.431	.223
32 F	1.50	2.00	1:1.33	1.0502	.6801	.6201	.3629	58.52	.459	.245
39 F	.75	1.00	1:1.33	.9478	.7286	.7172	.4134	57.64	.438	.249
40 M	.75	1.00	1:1.33	.9439	.6846	.6437	.3760	58.41	.393	.216
13 M	1.00	1.50	1:1.50	1.0246	.5942	.5504	.3156	57.21	.418	.222
14 F	1.00	1.50	1:1.50	1.0042	.6002	.5668	.3333	58.81	.434	.241
3 F	.50	.75	1:1.50	.8431	.5002	.4828	.2638	54.64	.400	.211
4 F	.50	.75	1:1.50	.9570	.5462	.5201	.2786	52.35	.395	.201
19 F	.50	.75	1:1.50	.5525	.4801	.4346	.2401	55.26	.363	.181
20 F	.50	.75	1:1.50	.4742	.3345	.3121	.1720	55.34	.334	.172
33 M	1.50	2.50	1:1.66	.9201	.5719	.5230	.3015	57.65	.476	.251
34 M	1.50	2.50	1:1.66	.9984	.6401	.5948	.3431	57.68	.400	.214
35 M	1.50	3.00	1:2	.9984	.6660	.6002	.3398	56.61	.438	.223
54 F	1.50	3.00	1:2	.8301	.5347	.4760	.2675	56.20	.424	.212
55 F	1.50	3.00	1:2	.8148	.5601	.4991	.2850	57.10	.400	.203
17 F	.25	.67	1:2.68	.5392	.2876	.2482	.1014	41.81	.355	.125
18 F	.25	.67	1:2.68	.4844	.2621	.2249	.0903	40.15	.312	.107
11 F	.50	1.50	1:3	1.0595	.6588	.6010	.3483	57.95	.470	.248
12 M	.50	1.50	1:3	1.0023	.6100	.5789	.3323	57.40	.448	.244
1 F	.25	.75	1:3	.9602	.5339	.4901	.2601	51.86	.375	.183
2 F	.25	.75	1:3	.8450	.4701	.4500	.2295	50.79	.361	.176
43 M	1.50	6.00	1:4	.6044	.4443	.3785	.2208	58.34	.634	.315
44 M	1.50	6.00	1:4	.7147	.5203	.4624	.2657	57.46	.605	.309

Table 3. Relation of lime and phosphoric acid in ration of weights of rats and to the weight of bone ash.

Group Grams	Ratio of $CaO:P_2O_5$ in ration	Lime in ration per cent	Phosphoric acid in ration per cent	Ratio Ca:P in ration	Weight of rats grams	Ash in extracted femur per cent	Dried femur in rat per cent	Femur ash in rat per cent	No. of rats
160–170	1: .25	6.00	1.50	1: .15	171	58.71	.434	.246	2
	1: .33	4.50	1.50	1: .20	161	57.70	.419	.240	2
	1:1.00	3.00	3.00	1: .61	161	59.28	.467	.253	2
	1:1.00	1.50	1.50	1: .61	160	58.61	.441	.238	4
	1:1.00	1.00	1.00	1: .61	170	60.79	.406	.229	2
	1:1.33	.75	1.00	1: .79	170	58.02	.415	.238	2
Average					166	58.85	.430	.239	
144–153	1: .50	3.00	1.50	1: .30	153	58.78	.455	.242	2
	1: .60	2.50	1.50	1: .36	145	59.77	.467	.270	2
	1: .60	1.50	1.00	1: .40	155	57.67	.474	.312	2
	1:1.00	.75	.75	1: .61	144	56.87	.453	.237	2
	1:1.50	1.00	1.50	1: .91	146	58.01	.426	.231	2
	1:1.66	1.50	2.50	1: .10	148	57.67	.438	.231	2
Average					148	58.12	.452	.253	
130–140	1:1.333	1.50	2.00	1: .79	138	56.63	.445	.234	2
	1:2.00	1.50	3.00	1:1.22	140	56.63	.420	.212	2
	1:3.00	.50	1.50	1:1.83	138	57.67	.459	.246	2
	1:3.00	.25	.75	1:1.83	136	51.32	.368	.179	2
Average					138	55.56	.423	.217	
78–125	1: .50	1.50	.75	1: .30	125	54.71	.438	.218	3
	1: .75	2.00	1.50	1: .45	99	51.65	.429	.196	2
	1:1.50	.50	.75	1: .91	124	58.01	.348	.176	4
	1:2.68	.25	.67	1:1.63	83	40.98	.333	.116	2
	1:4.00	1.50	6.00	1:2.44	78	57.90	.619	.312	2
Average					101	52.65	.433	.203	

average percentage of ash in the femurs was 58.85. The bones seemed to be normal in content of ash and in percentage of ash in the femur though the ash in the femur from the rat receiving 1.0 per cent each of lime and phosphoric acid was above normal (60.79 per cent).

The three rations with a lime-to-phosphoric-acid ration of 1 : 1 gave good growth and a normal percentage of femur ash. These rations contained 1.0 to 3.0 per cent of each mineral. The ration containing 0.75 per cent of lime and 1.0 per cent of phosphoric acid also gave normal growth and bones, as did the rations containing 4.5 per cent and 6.0 per cent of lime with 1.5 per cent of phosphoric acid and ratios of 1 : 0.33 and 1 : 0.25.

EFFECT OF DEVIATION FROM REQUIREMENTS FOR NORMAL GROWTH

The weights of the rats in the second group of Table 3 are below normal, though the deficiency is small in some cases. The ash in the femurs, while a little less than in the first group, could be termed normal and the same could be said regarding the ash content of the femurs. This indicates that slightly unfavorable percentages of lime and phosphosoric acid may not affect the structure or quantity of the bones, while they may decrease the growth of the animal.

The rats on the ration which contained 0.75 per cent of lime and 0.75 per cent of phosphoric acid (Group 2) weighed 144 grams, while the rats which received the same amount of lime and 1.0 per cent of phosphoric acid made normal growth (Group 1). This shows that less than 1.0 per cent of phosphoric acid or less than 0.75 per cent of lime is below the minimum for good growth, since this ratio of 1 : 1 has proven favorable for good growth and normal quantity of ash in the femurs when larger amounts of the minerals are fed.

Either decreasing the lime from 1.5 to 1.0 per cent in the ration when 1.5 per cent of phosphoric acid was present or increasing it to 2.5 per cent, decreased slightly the gain in weight of the rats. The final weights of the rats were 145 and 146 grams compared with 161 grams of the other rations.

The graph (Fig. 1) brings out the relation between the percentage of lime and phosphoric acid in the ration and the average weights attained by the rats. The average normal weights are in circles. Three of the normal weights are on rations with the ratio of lime to phosphoric acid of 1 : 1, and percentages of 1, 1.5, and 3. One of the normal weights is at the percentage of 0.75 : 1, and the two others at 4.5 : 1.5 and 6 : 1.5. The graphs show very clearly that small increases or decreases in either lime or phosphoric acid from the favorable amounts decrease the weights of the rats.

In the third group in Table 3 are shown the rats which attained a final average weight of 136 to 140 grams. The rats on the ration containing 1.5 per cent of lime and 2.0 to 3.0 per cent of phosphoric acid made practically the same growth (138 and 140 grams), while the per-

centages of ash in the femurs were the same. The ration containing 0.50 per cent of lime and 1.50 per cent of phosphoric acid produced rats with a final weight of 138 grams and with bones which contained 57.7 per cent of ash, while the ration which contained only 0.25 per cent of lime and 0.75 per cent of phosphoric acid produced rats with a final weight of 136 grams and bones which contained only 51.3 per cent of

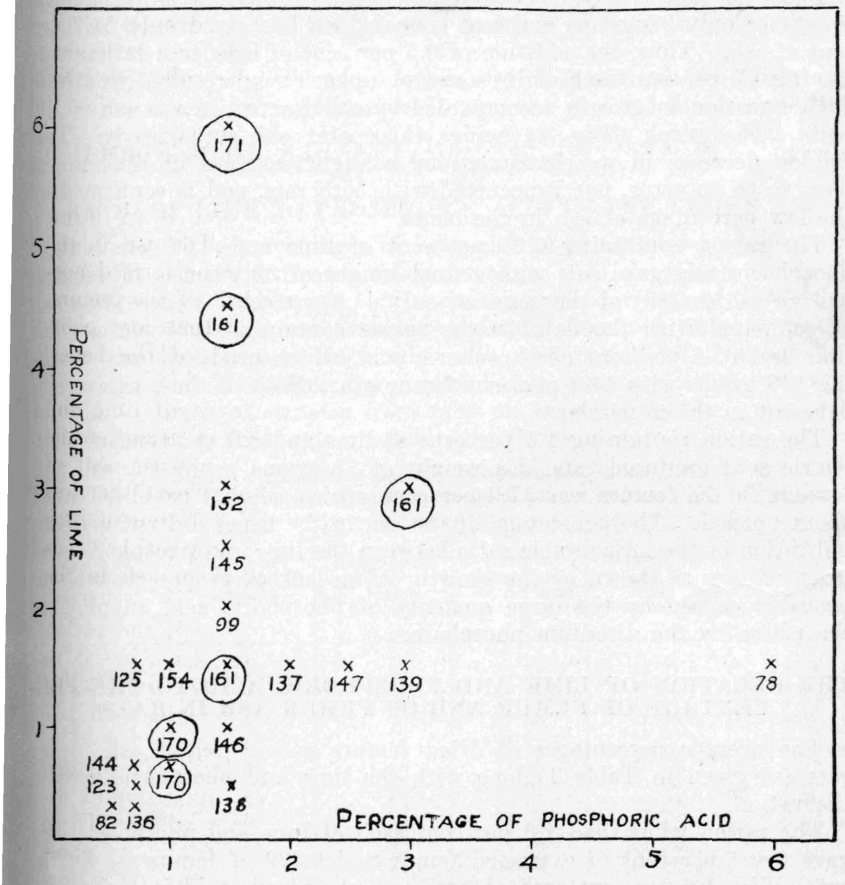

Figure 1.—Relation of weight of rats to percentages of lime and of phosphoric acid in the ration.

ash. It is probable that the low percentage of lime and phosphoric acid in the ration was the important factor in producing a bone with a low percentage of ash. It is probable that in the ration containing 0.25 per cent of lime and 0.75 of phosphoric acid, both lime and phosphoric acid are below the minimum, while in the ration containing 0.50 per cent of lime and 1.50 per cent of phosphoric acid, lime only is be-

low the minimum required for good growth and proper bone development.

AMOUNTS OF MINERALS WHICH ARE DISTINCTLY UNFAVORABLE

In the last group, Table 3, are placed all rats which attained a final weight of less than 135 grams. The rats on the ration containing 2.0 per cent of lime and 1.5 per cent of phosphoric acid, attained a final weight of only 99 grams and had bones which contained only 51.7 per cent of ash. Thus, the addition of 0.5 per cent of lime to a ration containing 1.5 per cent each of lime and phosphoric acid resulted in a serious depression in growth accompanied by a deficiency in the ash of the bone. The graph (Fig. 1) brings this point out very clearly. This sudden decrease in weight caused by a slight increase in lime might seem to be an error, but it occurred with both rats, and is confirmed by the low percentage of ash in the bones.

The ration containing 0.25 per cent of lime and 0.67 per cent of phosphoric acid gave rats with a final weight of 83 grams. These rats had an ash content of the bones of only 41.0 per cent. These data can be compared with the data on the ration containing 0.25 per cent of lime and 0.75 per cent of phosphoric acid, which produced rats weighing 136 grams with 51.3 per cent femur ash. Both of these rations are deficient in the minerals.

The ration containing 1.5 per cent of lime and 6.0 per cent of phosphoric acid produced rats of a weight of 78 grams, while the ash percentage in the femurs was 57.9 per cent, which is what could be called about normal. The percentage of the ash in the bones did not give any indication of the unfavorable ratio between the lime and phosphoric acid in the ration as shown by the growth. The lack of proper growth was probably caused by the large quantity of phosphoric acid supplied to the ration by the disodium phosphate.

THE RELATION OF LIME AND PHOSPHORIC ACID TO THE PERCENTAGE OF FEMUR AND OF FEMUR ASH IN RATS

The average percentages of dried femurs and of femur ash in the rats are given in Table 3 along with the lime and phosphoric acid in the ration.

The ration which had 1.0 per cent each of lime and phosphoric acid gave 0.407 per cent of extracted femur and 0.229 of femur ash in the rat. With 1.5 per cent each of lime and phosphoric acid in the ration, the rats contained 0.441 per cent of femur and 0.238 per cent of femur ash. These appear to be above the normal percentages.

The rats on a ration containing 0.50 per cent of lime and 0.75 per cent of phosphoric acid had only 0.348 per cent of femurs and 0.176 per cent of femur ash. The ration containing 0.25 per cent of lime and 0.67 per cent of phosphoric acid produced rats which had 0.333 per cent of femurs and 0.116 per cent of femur ash, although the aver-

age weight of the rats was only 82.5 grams. These low percentages of lime and phosphoric acid in the ration did not produce animals with a proper ratio of bone to the total weight of the rat.

The ration containing 1.5 per cent of lime and 6.00 per cent of phosphoric acid grew rats which had 0.619 per cent of femurs and 0.312 per cent of femur ash. If about 0.420 per cent of dried femur in the rat and about 0.230 per cent of femur ash is taken as normal, then the rats on this ration had too great a quantity of bone in proportion to the rest of the body.

All the other percentages of femur and of femur ash are fairly constant, not varying widely from 0.420 per cent of dried femur and 0.230 per cent of femur ash. Of course, there are many factors that affect the growth of rats as well as the bone content.

RATIO OF LIME TO PHOSPHORIC ACID IN THE FEMUR ASH

In Table 4 the percentages of lime and of phosphoric acid in a few samples of ash of the femurs are given along with the ration of lime to phosphoric acid. Although the percentages of lime and of phosphoric acid in the bone are not constant, the ratio does not range far from 1:1.2, which compares favorably with the results of Holmes and Pigott (8) and McCollum and Adams (12). There is some variation, but the variation of samples from rates on the same ration is almost as great as samples from rats on different rations.

Table 4. Relation of lime and phosphoric acid in the ration to the ratio of lime to phosphoric acid in femurs.

Ratio of $CaO:P_2O_5$ in ration	Lime in ration per cent	Phosphoric acid in ration per cent	Lime in femur ash per cent	Phosphoric acid in femur ash per cent	Ratio of lime to phosphoric acid in femur ash
1: .5	1.50	.75	53.37	42.37	1.26
1:1.0	1.50	1.50	49.78	39.50	1.25
1:1.0	1.50	1.50	49.30	40.28	1.22
1:1.0	.75	.75	47.56	41.44	1.12
1:1.0	.75	.75	51.83	41.03	1.23
1:1.50	1.00	1.50	44.03	39.26	1.12
1:1.50	1.00	1.50	41.83	37.98	1.10
1:1.50	.50	.75	56.01	45.00	1.24
1:1.50	.50	.75	53.51	44.54	1.20
1:3.0	.50	1.50	52.27	41.60	1.25
1:3.0	.50	1.50	53.05	41.74	1.27
1:3.0	.25	.75	49.40	42.33	1.17
1:3.0	.25	.75	53.34	44.71	1.19

There seems to be no relation between the ratio of lime to phosphoric acid in the ration fed and the ratio of lime to phosphoric acid in the bones.

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SUMMARY

Forty-nine rats in three series were fed rations containing fourteen different ratios of lime to phosphoric acid.

The percentages of lime in the rations ranged from 0.25 per cent to 6.00 per cent, while the percentages of phosphoric acid ranged from 0.67 per cent to 6.00 per cent. Ratios of lime to phosphoric acid varied from 1:.25 to 1:4.0. This is equivalent to a range of calcium-to-phosphorus ratio from 1:.15 to 1:2.44. Cod liver oil was fed to all rats.

Rations containing 1.0, 1.5, and 3.0 per cent of lime gave normal growth when the ratio to phosphoric acid was 1:1. Rations containing 4.5 or 6.0 per cent of lime and 1.5 per cent of phosphoric acid, also produced rats with a normal weight or above, and with femurs of a normal ash content. The minimum percentages which gave normal growth was found to be 0.75 per cent for lime and 1.0 per cent for phosphoric acid. Rations containing 0.25 per cent of lime and 0.75 per cent of phosphoric acid produced rats which had only 51.32 per cent of ash in the femur, and rations containing 0.67 per cent of phosphoric acid with 0.25 per cent of lime produced rats with only 40.98 per cent of ash in the femur. The normal percentage of ash in femurs was about 58.00 per cent. A ration containing 1.5 per cent each of lime and phosphoric acid gave normal growth, but when the percentage of lime was raised to 2.0, the growth was decidedly decreased and the percentage of ash in the bones was less than normal.

Percentages of lime or of phosphoric acid slightly lower than the most favorable percentages, in some cases decreased the growth, while the ash content of the femurs in some cases remained practically normal. Small increases in either lime or phosphoric acid from the favorable amounts in some cases decreased the growth, especially when the ration was low in minerals.

When the amounts of lime or phosphoric acid fed were below the minimum, the growth was decreased and the femur ash was lower than normal.

A percentage of 6.0 per cent of phosphoric acid in a ration with 1.5 per cent of lime caused a decided decrease in growth of the rats, but the percentages of ash in the femur was practically normal. The percentage of femurs in these rats was above normal.

No relation was found between the ratio of lime to phosphoric acid in the ration and the ratio of lime to phosphoric acid in the ash of the femur.

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