RESEARCH BULLETIN 567 OCTOBER, 1954

UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION
J. H. Longwell, Director

Effect on Guinea Pigs of Consuming An Excessive Quantity of Phosphates

A. G. HOGAN, W. B. HOUSE, AND W. O. REGAN

(Publication authorized October 20, 1954)

COLUMBIA, MISSOURI

Effect on Guinea Pigs of Consuming An Excessive Quantity of Phosphates

A. G. HOGAN, W. B. HOUSE, AND W. O. REGAN[1]

ABSTRACT

The colony ration supplied to guinea pigs in this laboratory has been reasonably satisfactory for growth and reproduction. It contains 1.2 percent of calcium and 0.9 percent of phosphorus. However, mature animals, over 18 months of age, at times develop sore and stiff joints, followed in some cases by visible deposits of calcium phosphate. If the amount of phosphorus in the ration is increased to 1.6 percent the deposits appear after about 19 weeks and presumably they will develop in every animal that survives the normal life span. A synthetic diet which contained approximately 1 percent of calcium and 1 percent of phosphorus was less suitable for guinea pigs than was the colony diet. The animals grew more slowly and there was a low incidence of calcium phosphate deposits when the animals were about 6 months old. When the phosphorus content of the diet was raised to 1.65 percent, the rate of growth was depressed still more, and half of the animals developed calcium phosphate deposits in an average time of 7 weeks. A small amount of evidence was obtained that the severity of the symptoms could be reduced by increasing the calcium content of the diet to 1.6, thus raising the calcium:phosphorus ratio to approximately 3:2.

INTRODUCTION

Hogan and Hamilton (1942) achieved a considerable degree of success in rearing guinea pigs on a semisynthetic diet. These studies were interrupted during the war and, when resumed, the earlier results could not be duplicated. In the meantime, the salt mixture incorporated in the diet had been changed to include a considerably larger amount of phosphorus, and it soon became apparent that this change explained the failure to obtain the same results. When the animals consumed the high-phosphorus diet they consumed less than the normal amount of food, grew more slowly, and they failed to reproduce. The wrists became sore and stiff, and after about five months swellings were observed in various parts of the body which were due to deposits of calcium phosphate. The stiff-wrist syndrome in guinea

[1]Supported in part by grants from the Office of Naval Research, U. S. Navy, and from the Frasch Foundation.

pigs was first described by Wulzen and Bahrs (1941). In the more severe cases deposits of calcium phosphate also developed. It has been shown that certain maladjustments in the intake of calcium and phosphorus by rats and by horses may be damaging to the bony structure, or they may precipitate deposits of calcium phosphate in soft tissues. The work at Missouri was resumed by Hogan, Regan and House (1950) who also reviewed the earlier literature. When the diet contained 0.8 percent of calcium and 0.5 percent of phosphorus there were only a few cases of calcium phosphate deposits and these developed when the animals were over one year old. However, when the amount of phosphorus was raised to 0.9 percent there were numerous cases of calcium phosphate deposits after much shorter experimental periods. The increase in the phosphorus content of the diet was not a complete explanation of the presence or absence of the abnormality but as far as we know this is the only explanation that has not been challenged. For some years this field of investigation was marked by extensive study and there were several reports that the condition was prevented or cured by some organic compound, presumably a vitamin, but this suggestion has not met with general acceptance. An extension of the observations of Hogan, Regan and House (1950) is described in the pages that follow.

EXPERIMENTAL PROCEDURES AND RESULTS

The experimental procedure was practically the same as the one described by Hamilton and Hogan (1942). Constituents of the rations that contained more than traces of mineral elements were analyzed for calcium, phosphorus, magnesium and potassium and the percentges of these minerals in the various diets were calculated from the analyses. It happened on a few occasions that a ration was changed in some minor manner which proved to have no significance. In such cases the combined results were ascribed to one ration only to save space and simplify the presentation.

SERIES I: Hogan, Regan and House (1950) had observed calcium phosphate deposits in guinea pigs when they consumed a synthetic type diet that contained an excessive amount of phosphorus. Colony diet No. 16, described in Table 1, contained as much phosphorus and considerably more calcium, than did Diet 713, described by Hogan *et al.,* (1950). In spite of its high phosphorus content, Ration 16 had been satisfactory as a colony

TABLE 1 -- RATION 16, FOR GUINEA PIG COLONY

Ground wheat	25 gm.	Steamed bone meal	2 gm.
Wheat middlings	10 gm.	Sodium chloride	1 gm.
Wheat germ	6 gm.	Dried yeast	15 gm.
Alfalfa meal	40 gm.	Vitamin A--D Conc.*	1 gm.
$MnSO_4 \cdot 4H_2O$	25 mg.	Ascorbic acid**	

*Vitamin A 1200 I.U., Vitamin D 120 I.U. per gm. of concentrate.
**10 mg. daily per animal, supplied separately.

diet and it seemed possible that the commonly used foodstuffs contained a nutrient that prevented the development of stiff joints, and of calcium phosphate deposits. To test that possibility, a new ration, No. 1993, was prepared as a modification of Ration 16. Ration 1993 contained approximately 1.1 percent of calcium and 1.6 percent of phosphorus and had a Ca:P ratio of 0.7:1. The results, and additional details concerning the rations, are shown in Table 2. Ration 1495, shown in Table 2, is practically identical with Ration 16, but since it was in use for only 12 weeks the results are shown separately.

TABLE 2 -- THE EFFECT OF AN EXCESSIVE INTAKE OF PHOSPHORUS BY GUINEA PIGS ON DEVELOPMENT OF CALCIUM PHOSPHATE DEPOSITS

Description of data		Ration 16		Ration 1495*		Ration 1993	
Variables in the diet							
Wheat middlings	gm.	10		10		7.9	
Steamed bone meal	gm.	2		2		0.5	
Sodium dihydrogen phosphate	gm.	0		0		3.6	
Protein	%	21.6		21.6		20.8	
Magnesium	%	0.25		0.25		0.24	
Potassium	%	1.77		1.77		1.71	
Calcium	%	1.2		1.2		1.1	
Phosphorus	%	0.9		0.9		1.6	
Ca:P ratio		1.3:1		1.3:1		0.7:1	
Observations on the animals							
Number and Sex		3M	3F	4M	4F	3M	3F
Avg. initial weight	gm.	172	178	178	161	175	180
Avg. daily gain	gm.						
1st 4 weeks		6.6	5.2	5.3	5.3	3.0	3.6
1st 8 weeks		6.2	4.8	5.6	5.4	3.4	2.7
1st 12 weeks		6.1	4.3	5.2	4.7	3.0	2.4
Stiffness and mineral deposits							
No. that became stiff		2		0**		6	
Avg. time for stiffness to develop, weeks		5, 47		----		16	
No. with calcium phosphate deposits		0		0		5	
Avg. time for deposits to develop, weeks		----+		----		19	

*100 grams of diet contains 354 I.U. of vitamin A, 50 I.U. vitamin D. Same as Ration 16 in all other respects.

**Checked for 12 weeks only.

+Some of the animals were checked for one year.

One of the 6 animals on Ration 16 developed stiffness in a period of 5 weeks, another developed stiffness in a period of 47 weeks, but this symptom was not observed in any of the other 4. None of the 8 animals on Ration 1495 developed stiffness but these were observed for only a short period. In contrast, all 6 animals on Ration 1993 developed stiffness within an average period of 16 weeks, and 5 of the 6 developed definite deposits of

calcium phosphate within an average period of 19 weeks. It will also be observed that the rate of growth on Ration 1993 was markedly depressed, to a level about one-half of that on the stock colony diet. There is no doubt that the addition of monosodium phosphate to Ration 16 makes it highly damaging to guinea pigs. At the time the experimental work was done we were unaware of the nutritional importance later ascribed to magnesium and potassium by Roine, Booth, Elvehjem and Hart (1949) who reported that guinea pigs do not attain maximum growth rates unless their diets contain liberal amounts of magnesium and potassium. Because of the importance now attached to these minerals we have calculated from recent analyses their percentages in the rations described in Table 2. In all probability the relatively high levels in Ration 1993 prevented a more drastic depression of the rate of growth. They certainly did not prevent the deposits of calcium phosphate though they may have delayed their appearance.

Aged Guinea Pigs and Calcium Phosphate Deposits

Since there were some examples of stiff wrists on Ration 16, six of the older animals were retained in the colony to see if deposits of calcium phosphate would eventually develop in aged animals. As is shown in Table 3,

TABLE 3 -- DEVELOPMENT OF CALCIUM PHOSPHATE DEPOSITS IN AGED ANIMALS THAT CONSUMED THE COLONY DIET NO. 16

Animal No.	Age When Deposits Were Detected (days)	Site of Deposits
448	583	Ribs, stomach
621	881	Ribs, shoulder, stomach, duodenum.
589	951	Muscles, stomach, duodenum.
582	1033	Leg
35	1062	Foot-pad
109	----	None at 653 days.

five of the animals did develop deposits, at ages varying from 583 to 1,062 days. The sixth animal died at the age of 653 days but no deposits could be detected. The appearance of calcium phosphate deposits in aged animals on Ration 16 presumably is due to some maladjustment in its composition and one would expect the diet to be improved by reducing its content of phosphorus. It seems clear that if Ration 16, or Ration 1993, contains a nutrient that prevents the appearance of calcium phosphate deposits, it is of a low order of effectiveness.

SERIES II: It seemed probable that synthetic rations would be more useful for our purpose than would Diet 16 so they were used in our later investigations. The percentage composition of the basal diet, No. 903, was: Casein,[2] 20; sucrose, 35; cellulose,[3] 15; lard, 10; salts,[4] 5; dried yeast 15. Two

[2]Prepared by the method of McCollum et al. ('22).
[3]Celluflour, Chicago Dietetic Supply House, Inc., Chicago, Illinois.

thousand I. U. of vitamin A, 293 I. U. of vitamin D, 2.5 mg. of vitamin E, and 2.5 mg. of menadione were incorporated in the 10 grams of lard. Vitamin C was supplied separately three days per week, at a level equivalent to 10 mg. daily per animal. The other diets of this series, and the results, are described in Table 4.

TABLE 4 -- THE EFFECT OF VARYING INTAKES OF CALCIUM AND PHOSPHORUS BY GUINEA PIGS ON THE DEVELOPMENT OF CALCIUM PHOSPHATE DEPOSITS

Description of data		Ration 903		Ration 1237		Ration 1238	
Variables in the diets							
Ration 903	gm.	100		97.25		98.25	
$NaH_2PO_4 \cdot H_2O$	gm.	-----		2.75		-----	
$CaCO_3$	gm.	-----		-----		1.75	
Magnesium	%	0.08		0.08		0.08	
Potassium	%	0.72		0.70		0.71	
Calcium	%	1.02		0.93		1.67	
Phosphorus	%	1.08		1.65		1.06	
Ca:P ratio		0.94:1		0.57:1		1.6:1	
Observations on the animals							
Number and Sex		22M	9F	45M	17F	11M	5F
Avg. initial weight	gm.	202	188	187	193	200	319
Avg. daily gain	gm.						
1st 4 weeks		3.0	1.7	2.9	1.7	4.5	1.7
1st 8 weeks		4.2	1.8	2.6	2.4	4.1	1.6
1st 12 weeks		3.4	0.8	2.3	2.1	3.5	1.4
Stiffness and mineral deposits							
No. of animals checked		15		41		4	
No. that became stiff		11		40		1	
Avg. time for stiffness to develop, weeks		8		6		20	
No. with calcium phosphate deposits		2		30		0	
Avg. time required for deposits to develop, weeks		26		10		--	
Mortality	%						
1st 4 weeks		0		0		0	
1st 8 weeks		3		5		0	
1st 12 weeks		26		15		0	

Ration 903 contains approximately 1 percent each of calcium and phosphorus, and is practically identical with Ration 713, described in a previous publication (Hogan *et al.,* 1950). The results are similar also, for a considerable proportion of the animals on Ration 903 developed stiff joints, and two of them developed deposits of calcium phosphate in an average period of 26 weeks. Ration 1237, contains considerably more phosphorus than calcium, and it produces severe damage in a comparatively short time. Of the animals that consumed it, 41 were checked and deposits were found on 30. Ration 1238 contains approximately 1 percent of phosphorus and 1.67 percent of calcium. This ration was supplied to only a few animals

but they were damaged less severely than were those on Ration 1237, for example. Only one animal on Ration 1238 developed stiff joints and none developed detectable deposits of calcium phosphate. It was assumed at the time that guinea pigs were unable to tolerate a large intake of phosphorus, and the assumption may be correct. However, a few scattered preliminary observations suggest that the guinea pig cannot tolerate an excess of various anions and that phosphate is not peculiar in this respect. We expect to return to this phase of the investigation.

DISCUSSION

The data show that unsuitable amounts, or possibly ratios, of calcium and phosphorus are disastrous to guinea pigs. They also show that there are interrelations among the nutrients which modify some of the effects. The colony ration, No. 16, is reasonably satisfactory for guinea pigs, but deposits of calcium phosphate did appear in aged animals. This appearance may be an unavoidable consequence of age. However, if unsuitable adjustments of the mineral intake hasten the development of calcium phosphate deposits one might expect suitable adjustments to delay them. All three of the rations in Table 2 contained considerable amounts of magnesium and potassium. This probably explains why the animals that consumed them were not affected more severely. Ration 903, in Table 4, contains approximately the same amounts of calcium and phosphorus as does Ration 16, but it contains less magnesium and potassium. The growth rate was lower than on Ration 16 and deposits of calcium phosphate developed comparatively soon. Ration 1237 contained approximately the same amounts of the basic elements as Ration 903, but it contained a much larger quantity of phosphorus. The deposits appeared much more quickly in these animals. We regard it as certain that all animals on Ration 1237 will develop deposits, if they do not meet an untimely death. Ration 1238 contains much more calcium than does Ration 903 but these two rations contain approximately the same amounts of phosphorus, magnesium and potassium. The ration with the higher calcium content was much more suitable for guinea pigs.

SUMMARY

An earlier report was confirmed which stated that guinea pigs develop deposits of calcium phosphate if they consume an excessive amount of phosphorus.

The deposits developed more quickly on synthetic diets than on the colony diet. This may be due to the higher percentages of magnesium and potassium in the latter ration.

There was no definite evidence that natural feedstuffs contained an organic nutrient that prevented the development of calcium phosphate deposits.

LITERATURE CITED

Hogan, A. G. and J. W. Hamilton, 1942. Adequacy of simplified diets for guinea pigs and rabbits. J. Nutrition, 23:533-543.

Hogan, A. G., W. O. Regan and W. B. House, 1950. Calcium phosphate deposits in guinea pigs and the phosphorus content of the diet. J. Nutrition, 41:203.

McCollum, E. V., Nina Simmonds, P. G. Shipley and E. A. Parks, 1922. Studies on experimental rickets. XXII. Conditions which must be fulfilled in preparing animals for testing the anti-rachitic effect of individual foodstuffs. Johns Hopkins Hosp. Bull., 33:296-302.

Richardson, L. R. and A. G. Hogan, 1946. Diet of mother and hydrocephalus in infant rats. J. Nutrition, 32:459-466.

Roine, Paavo, A. N. Booth, C. A. Elvehjem and E. B. Hart, 1949. Proc. Soc. Exp. Biol. Med., 71:90-91.

Wulzen, Rosalind and Alice M. Bahrs, 1941. Effect of milk diets on guinea pigs. Am. J. Physiol., Proc., 133:500.