Effect of a High Phosphorus Diet on Acid-Base Balance in Guinea Pigs.* (22218)

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The injurious effect of high phosphorus diets for the guinea pig has been reported (1,2). The animals grew slowly, developed mineral deposits in soft tissues and usually succumbed within a few weeks. More recently the ameliorative effect of magnesium and potassium on this syndrome has been described(3). Thus the guinea pig seems to be more sensitive to high levels of dietary phosphorus than the white rat and appears to have a considerably higher requirement for potassium and magnesium.

This report is concerned with one reason for the injurious effect of high phosphorus intake and the beneficial effects of potassium and magnesium.

Methods. The diets used were similar to those described by House and Hogan(3). The percentage composition of the basal ration (3884) was: acid-washed casein(4) 30 g; sucrose 47; celluflour 15; soybean oil 4; salts‡ 4. The vitamins§ added per 100 g of diet were: thiamine HCl 1 mg; riboflavin 1 mg; pyridoxine HCl 1 mg; calcium pantothenate 3 mg; niacin 5 mg; choline chloride 100 mg; inositol 100 mg; folic acid .6 mg; biotin .02 mg; vit B_{12} .003 mg; ascorbic acid 50 mg; alpha tocopherol 2 mg; 2-methyl-1, 4-naphthoquinone 1 mg; vit. A 2000 I.U. and vit D 285 I.U. In some diets gum arabic was substituted for celluflour, but since it had only a slight effect on the acid-base balance, data for gum arabic and celluflour diets were combined. The basal diet contained about 0.4% of phosphorus and 0.9% of calcium. The salt mixture of Richardson and Hogan (6) was used at a level of 5% to give a phosphorus content of 0.9% and $NaH_2PO_4 \cdot H_2O$ was added to give a phosphorus content of 1.7%. Magnesium was added as MgO, potassium and sodium as acetates, and calcium as the carbonate. All additions were made at the expense of sucrose. Diets were analyzed for calcium, magnesium, sodium, potassium and phosphorus, and are reported to the nearest tenth of a percent. Chloride and sulphur contents of diet were calculated from the composition of the salts mixture and the protein. For the purpose of calculating the acid-base balance of diets the method of Shohl and Sato(7) was employed except the results are recorded as milliequivalents of excess cations per 100 g. For growth studies guinea pigs weighing 175 to 225 g were fed *ad libitum* for 8 weeks. Most of blood and urine studies were made at the end of this period, but in the case of more severe rations, very few animals survived 8 weeks. For blood studies on these rations older animals were used and an adjustment period of 2 to 4 weeks was allowed before samples were taken. Blood for carbon dioxide capacity was taken by cardiac puncture while animals were under ether anesthesia and the determination was made on plasma by the manometric method of Van Slyke(8). Urine samples for both pH and ammonia determinations were removed directly from the bladder by catherization of anesthetized animals. The pH was determined immediately by the glass electrode and urinary ammonia determined on 5 ml of pooled sample by the method of Van Slyke and Cullen(9). Blood for determination of inorganic phosphorus was collected by clipping a clean toe nail and a 0.1 ml sample was immediately hemolyzed in water. Within 30 minutes the sample was deproteinized with

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‡ The salts mixture described by Hubbell *et al.*,(5) to which was added 2.5 g of $MnSO_4 \cdot 4H_2O$/100 g.

§ Folic acid was supplied by Lederle Laboratories, Pearl River, N. Y., biotin by Hoffmann-LaRoche, Nutley, N. J., and other crystalline vitamins by Merck and Co., Rahway, N. J.

TABLE I. Effect of Mineral Composition of Diet on Rate of Gain, Blood Phosphorus, Urine pH and Plasma Carbon Dioxide Capacity.

Ration No.	Variable constituents* P	Na	K	Mg (%)	Excess cations per 100 g, mEq	Daily gain (8 wk),† g	Whole blood P, mg/100 ml	Urine pH	Plasma CO_2 cap., vol %
3884	.4	.1	.4	.1	21	3.2 (11)‡	6.0 (11)	7.3 (17)	50 (10)
3480	.9	.3	.4	.1	1	1.6 (14)	8.1 (23)	5.9 (11)	50 (3)
3475	1.7	.8	.4	.1	−20	.5 (6)	8.6 (36)	5.6 (13)	34 (10)
3479	.4	.1	1.5	.4	74	4.7 (18)	5.3 (20)	8.2 (20)	48 (14)
3515	.9	.3	1.5	.4	53	3.5 (8)	5.3 (28)	7.8 (8)	50 (6)
3477	1.7	.8	1.5	.4	32	3.8 (16)	7.5 (55)	7.2 (25)	46 (21)
16§	.9	.3	1.8	.25	—§	5.2 (24)	5.0 (58)	8.1 (11)	49 (18)

* Calcium content of all diets was 0.9% except No. 16 which contained 1.5%.
† Avg of mean daily gains of males and females.
‡ No. of animals on which avg is based is shown in parentheses.
§ Ration 16 was a stock diet composed of natural feedstuffs. Analysis was incomplete.

trichloroacetic acid and the method of King (10) was followed.

Results. The effects of different levels (0.4, 0.9 and 1.7%) of dietary phosphorus on rate of gain, blood inorganic phosphorus, urine pH and plasma carbon dioxide capacity are shown in Table I. The levels of calcium (0.9%), potassium (0.4%) and magnesium (0.1%) are those commonly used in purified diets for rats and chicks and these animals thrive on such diets even with calcium to phosphorus ratios as close as 1:1. However, in the guinea pig rate of growth was subnormal even at a low level of phosphorus (3884) unless the diet was supplemented with potassium and magnesium (3479). With higher levels of phosphorus rate of gain became progressively less until at a level of 1.7% most animals succumbed and survivors grew extremely slowly. Blood phosphorus rose to an average of 8.6 mg%, urine pH dropped to 5.6, and plasma carbon dioxide capacity dropped to 34 vol. %. These values should be compared with those obtained on animals fed the stock diet (16) composed of commonly used foodstuffs. The data show a positive correlation between rate of growth and urine pH and a negative correlation between rate of growth and blood inorganic phosphorus. It is obvious that the high phosphorus diet (3475) is an acid diet not only from the calculated deficit of cations, but also from its effect on urine pH and plasma carbon dioxide capacity. Addition of potassium and magnesium to the diets at all levels of phosphorus markedly improved growth, lowered blood phosphorus and raised urine pH. Addition of potassium and magnesium to a ration which contained 0.9% of phosphorus (3515), gave a nearly normal blood and urine picture. Addition of these cations to the excessively high phosphorus ration (3477) improved the rate of growth and raised urine pH, but did not maintain normal blood phosphorus and urine pH values. Thus, the beneficial effect of high levels of potassium and magnesium in the diet of the guinea pigs is due partly, but not wholly, to the maintenance of a positive base balance and a normal level of blood phosphorus.

Of the indices studied the urine pH seemed

TABLE II. Effect of Various Cations on the Urine pH of Guinea Pigs Fed a High Phosphorus Diet.

Ration No.	Additions: Cation	%	mEq per 100 g	Urine pH	Daily gain* (8 wk)
3475	None	—	—	5.6 (13)†	.5 (6)
3895	K‡	1.1	28	6.5 (9)	.9 (10)
4120	K‡	2.2	56	7.4 (8)	2.0 (4)
3780	Na§	.6	26	6.7 (6)	1.7 (6)
3889	Na‡	.5	22	6.3 (4)	.7 (14)
3897	Na +	1.0	44	7.6 (6)	3.8 (7)
	Mg	.3	25		
3477	K +	1.1	28	7.2 (25)	3.8 (16)
	Mg	.3	25		
3894	Mg	.3	25	6.4 (9)	2.1 (9)
4119	Mg	.6	50	7.0 (11)	2.1 (4)
3904	Ca	1.6	80	6.8 (9)	2.0 (8)

* Avg of mean daily gains of males and females.
† No. of animals shown in parentheses.
‡ Sodium and potassium were supplied as acetate.
§ Sodium was supplied as Na_2HPO_4 which replaced $Na_2HPO_4 \cdot H_2O$ in the diet.

TABLE III. Urinary Ammonia Excretion in the Guinea Pig.

Ration No.	Excess cations per 100 g, mEq	Urinary NH_3-N, mg/100 ml	Urine pH
3884	21	1.1 (2)*	7.3 (17)
3479	74	.9 (2)	8.2 (20)
3475	−21	1.1 (3)	5.6 (13)
16	—	1.4 (3)	8.1 (11)
Human urine	—	60.0 (2)†	6.5 (2)

* No. in parenthesis indicates No. of determinations. Urine from several animals was pooled for a determination.

† These values were determined on freshly voided samples and hence do not represent a 24 hr specimen, but they are in the range commonly given for normal human urine.

to be the most sensitive to slight changes in acid-base balance. Hence, it was used to study the relative value of various cations in counteracting the injurious effects of a high phosphorus diet. Results of the growth studies and urine pH determinations are shown in Table II. Addition of each of the cations studied, potassium, sodium, magnesium and calcium, improved rate of growth and raised the urine pH. Within limits, the cations were of about equal efficacy and the effect was in proportion to the quantity added. Although potassium is more abundant than sodium in the natural diet of herbivorous animals, as a supplement to the basal diet, sodium appeared as effective as potassium either alone or in combination with magnesium. On an equivalent basis none of the cations was as effective alone as in combination. Calcium ion was quite effective in raising urine pH because in the guinea pig a relatively high proportion of the calcium is excreted by way of the kidneys (unpublished data).

The guinea pig like the rabbit(11) is more sensitive to an acid diet than most animals chiefly because it does not use ammonia to neutralize excess acids excreted by way of the kidney. In Table III are shown results of ammonia nitrogen analyses of fresh urine samples. These values on guinea pig urine agree very well with those reported for rabbit urine(12) but are of the order of 2% of the ammonia commonly found in human urine. Urine from animals fed a high phosphorus diet had a low pH but the amount of ammonia was approximately the same as that found in urine of a high pH. Thus, consumption of an acid diet caused a change from basic to acid phosphates in the urine, but it did not stimulate the excretion of urinary ammonia. Guinea pigs that consume a stock colony diet normally excrete an alkaline urine which is quite turbid. Qualitative analysis of the insoluble portion indicates that it is composed chiefly of calcium and magnesium phosphate and microscopic examination shows that phosphates are held in a noncrystalline, colloidal state. The urine excreted when animals consume a high phosphorus diet, is not turbid but is clear in appearance.

Since there is a negligible amount of ammonia excreted in the urine of the guinea pig, the shift to an acid urine appears to be the chief mechanism for conserving fixed bases. This mechanism was effective in maintaining a normal plasma carbon dioxide capacity when the level of dietary phosphorus was 0.9%, but at a level of 1.7% of phosphorus there was marked drop in carbon dioxide capacity. It seems possible that high blood phosphorus and the incidence of soft tissue calcification observed when animals consume a high phosphorus diet that is relatively low in potassium and magnesium are due in part to the inability of the guinea pig to excrete phosphorus normally under the conditions of a low base reserve.

Summary. Injurious effects of high phosphorus diets for the guinea pig, which include poor growth, metastatic calcification, and death, are due in part to the fact that this animal does not tolerate an acid diet. This has been demonstrated by the marked drop in urine pH, a decrease in plasma carbon dioxide capacity and an increase in blood inorganic phosphorus. Addition of various cations produced a more alkaline urine, but a combination of sodium or potassium and magnesium proved most beneficial. The guinea pig is highly sensitive to an acid diet because a negligible amount of ammonia is excreted by way of the kidneys.

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A Histamine-Like Component of Commercial Pitressin.* (22219)

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Several investigators have assigned an important role to pitressin as a releasing agent for ACTH. Thus, Mirsky, Stein and Paulisch(1), suggested that the antidiuretic hormone of the posterior pituitary (ADH, Vasopressin) may serve as a mechanism for ACTH release under stress. McCann and Brobeck (2), also implicated ADH as a factor for release of corticotropin. They destroyed the supraoptico-hypophyseal tract thereby inhibiting the usual output of ACTH in response to stress. However, when animals bearing hypothalamic lesions were injected with commercial pitressin, ACTH was released. Guillemin and Hearn(3), employing rat pituitaries and a tissue culture technic, demonstrated that pitressin releases ACTH from the adenohypophysis *in vitro,* whereas highly purified arginine-vasopressin obtained from du Vigneaud was without effect. The ACTH releasing activity of pitressin was attributed to an unknown contaminant probably originating in the hypothalamus. Saffran, Schally and Benfey(4), also reported that vasopressin contained a corticotropin-releasing factor as an impurity which they were able to separate from vasopressin by paper chromatography.

In view of reports(5-7) that extracts prepared from posterior pituitary powder and fresh glands contain histamine, it was considered possible that minute amounts of the amine bound to the polypeptide were carried through the extraction procedure for commercial pitressin and persisted in the finished product. To anticipate, it can be stated that commercial pitressin contains a component of unknown nature which exhibits some of the physiological characteristics of histamine and which for brevity we shall hereafter refer to as "histamine-like."

Methods. Pitressin† (Vasopressin Injection, 20 units/cc, Parke, Davis and Co.) was extracted with strong acid at boiling temperature. The material was pooled in batches containing 3200 units, in approximately 160 cc, and sufficient concentrated HCl added to make an 18% solution. The fluid was divided among 3 flasks equipped with water cooled reflux condensers and the pitressin boiled vigorously for 1 hour. The commercial material is preserved with 0.5% chlorobutanol which readily crystallizes on the condenser and was removed. After 1 hour the water condensers were replaced by reflux air con-

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