THE COMPARATIVE UTILIZATION OF ORTHO AND PYRO PHOSPHATE IN BONE DEVELOPMENT OF RATS

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THE COMPARATIVE UTILIZATION OF ORTHO AND PYRO PHOSPHATE IN BONE DEVELOPMENT OF RATS

by

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HISTORICAL

Ortho phosphoric acid and the various salts of this acid are known to supply phosphorus for the various needs of the body, the major ones being for the normal development of bones and teeth. Thus for example ortho phosphates are selected to supply the major portion of the phosphorus of synthetic experimental diets. In addition to these sources foodstuffs, of course, contain much of their phosphorus in the form of phosphorylated organic compounds which on hydrolysis yield ortho phosphate.

Pyro phosphates have been used for over twenty years as an ingredient of leavening agents and interest in their wholesomeness has recently been aroused in connection with the revisions in federal food and drug regulations. A persual of the literature reveals that relatively little work has been done on the study of the fate of pyrophosphates in the animal body. Gamgee, Priestly, and Larmouth[1] assumed that pyrophosphate passes unchanged through the body and by intravenous injections of the normal salt into rabbits and frogs demonstrated that the pryophosphate has a definite toxic effect, causing a fall in blood pressure and a variation in pulse rate, with continuous injections leading to death. However, they do note the following: "We have never succeeded in producing symptoms of poisoning by sodium pyro phosphate when we have introduced the substance into the stomach. In dogs the drug has produced vomiting if fed in large amounts but no further ill effect; rabbits have remained uneffected by it. So striking was the absence of poisonous effects that it appeared to us possible that some of the alimentary ferments might cause sodium pyro phosphate to combine with water and convert it to the inert ortho phosphate. Direct experiments with salivary, gastric, and pancreatic ferments digested with pyro phosphate at the temperature of the body, proved that our surmise was not correct. At present we are inclined to think that the fact of animals not being poisoned when sodium pyro phosphate is introduced into the alimentary tract is due to the rapid elimination of the drug." In comparison Gamel[2], in 1911, noted that the pyro phosphate goes over to the ortho phosphate in an organism. Gardner and Symes[3] working with cats demonstrated that a small intravenous injection of the tetra sodium pyro phosphate causes a fall in arterial pressure and a slightly larger dose proves fatal. It was at one time thought that the injurious effect of cotton seed oil was due to the presence of pyro phosphate.[4] In this work the supposed harmfulness of the pyro phosphate was demonstrated by injecting an extract of the cotton seed meal into the blood stream of a rabbit. Later investigations showed that the oral administration of this same extract had no effect on the animals.

Starkenstein[5] believed that the conversion of pyro phosphate to ortho phosphate takes place in the stomach, due to the presence of hydrochloric acid. Data obtained from the Victor Chemical Works indicates that the hydrolysis of pyro phosphate in the presence of .1 N hydrochloric acid proceeds at a relatively slow rate, less that 70% being converted to ortho phosphate in a period of eight days. Inasmuch as food rarely remains in the stomach for more than six to eight hours, it is obvious that relatively little conversion would take place in this region of the digestive tract.

Lohman[6] has determined that the pyro phosphate is transformed to the ortho phosphate in an organism, although the site of such change was not suggested.

On the basis of experimental data of Behrems and Seelkoph[7] who fed sodium pyro phosphate to male mice it was demonstrated that ingestion of the pyro phosphate added as the salt to the diet caused no harm to the animals. Hoppert[8] showed that pyro phosphate added to an experimental diet exerted no abnormal influence on the development of the rat and on reproduction and rearing of its young.

In view of the limited and inconclusive studies reported in the literature on the utilization and fate of pyro phosphates in the animal body it was considered desirable to do further work to determine whether ingested pyro phosphate is converted to and used as ortho phosphate.

EXPERIMENTAL PART I

In order to demonstrate the conversion of pyro phosphate to ortho phosphate a comparison was made of the relative effects of equivalent amounts of the basic and acid pyro and ortho phosphates when added to a ricketogenic diet. The use of such a diet in the production of experimental rickets is based on the fact when the phosphorus of the diet is limited in amount and restricted to cereal sources in which much of the phosphorus is in the form of phytin, the addition of a large amount of calcium carbonate will so decrease utilization of phosphorus that calcification of the skeleton is practically entirely inhibited. Blood phosphorus values drop to an exceedingly low level. If to such a diet available phosphorus such as sodium ortho phosphate is added, there is an immediate response in blood phosphorus values and in bone development.

The method employed therefore was simply to compare the effects of equivalent amounts of the basic and acid salts of pyro and ortho phosphoric acid when supplemented to a basal ricketogenic diet and fed to young growing rats.

Young rats, 21 to 25 days old and weighing between 45 and 50 grams, were selected and divided into groups as whown in Table I. The basal diet used in these studies had the following composition:

Ground yellow corn --------	1605 grams
Ground oatmeal ------------	600 grams
Wheat gluten --------------	600 grams
Yeast ---------------------	125 grams
Calcium carbonate ---------	90 grams
Sodium chloride -----------	30 grams

The animals were fed their respective diets for a period of seven weeks. At the end of this period they were killed, blood samples collected, and the femurs removed.

The blood phosphorus value of each animal was determined by the Youngberg procedure using the Fisher Electrophotometer in place of the colorimeter. The percent bone ash of the femur of each animal was determined in the conventional manner, i.e., on the fat extracted dessicated bones. The analytical results obtained in this study are summarized in Table II. The detailed data are assembled at the end of the thesis.

DATA:

TABLE I

Supplements to the Ricketogenic Diet

Group No.	No. of Animals	Supplement	Amount P contributed by supplement per 100 gm. of ration
1	6	none	---
2	6	$CaCO_3$ reduced to 1%	---
3	6	$Na_3PO_4 \times 12H_2O$	164 mg.
4	6	$Na_3PO_4 \times 12H_2O$	82 mg.
5	6	$Na_3PO_4 \times 12H_2O$	41 mg.
6	6	$Na_4P_2O_7 \times 10H_2O$	164 mg.
7	6	$Na_4P_2O_7 \times 10H_2O$	82 mg.
8	6	$Na_4P_2O_7 \times 10H_2O$	41 mg.
9	6	$NaH_2PO_4 \times H_2O$	246 mg.
10	14	$NaH_2PO_4 \times H_2O$	164 mg.
11	8	$NaH_2PO_4 \times H_2O$	82 mg.
12	7	$NaH_2PO_4 \times H_2O$	41 mg.
13	7	$Na_2H_2P_2O_7 \times H_2O$	246 mg.
14	7	$Na_2H_2P_2O_7 \times H_2O$	164 mg.

TABLE II

Average Bone Ash and Blood Phosphorus Values

No. of animals	Phosphorus source	Amount P contributed by supplement per 100 gm. of ration	Percent bone ash	Blood P (mg./100ml of blood	Weight (seven weeks)
6	Basal	None	17.55	2.7	77
6	Basal with only 1% calcium CO_3		38.72	4.99	124
6	$Na_3PO_7 \times 12H_2O$	164 mg.	40.75	7.57	116 gm.
6	$Na_3PO_7 \times 12H_2O$	82	31.43	5.90	119
6	$Na_3PO_7 \times 12H_2O$	41	25.06	3.70	104
6	$Na_4P_2O_7 \times 10H_2O$	164	33.85	7.06	118
6	$Na_4P_2O_7 \times 10H_2O$	82	25.25	4.23	115
6	$Na_4P_2O_7 \times 10H_2O$	41	20.03	3.31	99
6	$Na\ H_2PO_4 \times H_2O$	246	39.95	6.80	114
14	$Na\ H_2PO_4 \times H_2O$	164	38.18	5.72	113
8	$Na\ H_2PO_4 \times H_2O$	82	31.22	4.68	105
7	$Na\ H_2PO_4 \times H_2O$	41	21.85	----	93
7	$Na_2H_2P_2O_7 \times H_2O$	246	41.02	6.75	124
7	$Na_2H_2P_2O_7 \times H_2O$	164	38.19	5.45	116

Discussion:

An examination of Table II discloses in general that all additions of ortho or pyro phosphates caused a definite increase in bone ash, blood phosphorus, and the weight of the animal. Group 2 was included to demonstrate the effect of lowering the calcium carbonate content of the diet.

By comparing the average results of the alkaline salts with the basal diet it may be readily observed that the addition of increasing amounts of either the tetra sodium pyro phosphate or the tri sodium ortho phosphate caused a definite improvement in bone development as indicated by the increases in percent bone ash. As might be expected the blood phosphorus values showed corresponding increases.

Comparison of the results obtained by feeding the tri sodium ortho phosphate with those of the tetra sodium pyro phosphate indicate that the alkaline salts of the pyro phosphate are not utilized as well as the comparable ortho phosphates. This conclusion is based on the fact that the bone ash and the blood phosphorus values were consistently lower in the case of the tetra sodium pyro phosphate. Nevertheless, the tetra sodium pyro phosphate was appreciably utilized in every instance as shown by the increased bone ash as well as the blood phosphorus values when compared with the basal group. In contrast the test with the acid salts reveal striking similarity in effects at all levels.

This indicates the complete utilization and conversion of the acid pyro phosphate to the ortho phosphate form. Inasmuch as the acid pyro phosphate employed in baking is the disodium dihydrogen pyro phosphate and reacts with sodium bicarbonate to form predominately the trisodium monohydrogen phosphate, one may assume that the responses with the latter would at least fall between the limits extablished for the tetra and the disodium pyro phosphate. It would accordingly be reasonable to conclude that the use of disodium dihydrogen pyro phosphate in baking contributes appreciably to the amount of useful phosphorus in a food prepared with an acid pyro phosphate leavening agent.

On the basis of these results one is permitted to conclude that the pyro phosphates are well utilized for maintaining a normal blood phosphorus level and supporting bone development. It is reasonable to assume that all of the other uses for phosphorus may likewise be served by ingested pyro phosphates.

EXPERIMENTAL PART II

Since it was found (Part I) that the pyro phosphate can be well utilized in bone development and claims in the literature have been made that the pyro phosphate is converted to the ortho phosphate before it reaches the blood, it was desired to determine whether the method employed for the analysis of blood was reasonably specific for ortho phosphate. Accordingly solutions of both ortho and pyro acid phosphate containing .1 mg. of P per ml. were made and the phosphorus content of each solution was determined according to the Youngberg procedure for blood phosphorus. The following results were obtained:

Phosphorus Source	Actual phosphorus content	Analyzed P content
Socium acid ortho phosphate	8 mg./100 ml.	8 mg./100 ml.
Sodium acid pyro phosphate	8 mg./100 ml.	.6 mg./100 ml.
Sodium acid pyro phosphate	12 mg./100 ml.	.7 mg./100 ml.

From the above it is readily observed that the reaction between pyro phosphate and the Youngberg reagent is very slight.

The small amount determined in the analysis of the pyro phosphate solution may be due to the conversion of the pyro to the ortho form by hydrolysis. Upon analysis of the above solutions at later dates it was found that the amount of phosphorus gradually increased. At the end of ten days there was about fifty percent conversion to the ortho form.

It was desired, also, to determine if the trichlor-acetic acid used in precipitating the proteins in this method hastened the conversion of the pyro to the ortho form. The following shows the results obtained:

S.A.P.P. (1 ml. N. 1 mg. P)	T.C.A.A. added	Analyzed P content (1 hr. after mixing)	Analyzed P content (4 days after mixing)
10 ml.	80 ml.	.8 mg./100 ml.	2.9 mg./100 ml.
10 ml.	40 ml.	.8 mg./100 ml.	2.8 mg./100 ml.
10 ml.	20 ml.	.8 mg./100 ml.	2.76mg./100 ml.
10 ml.	10 ml.	.8 mg./100 ml.	2.7 mg./100 ml.
10 ml.	5 ml.	.8 mg./100 ml.	2.61 mg/100 ml.
10 ml.	none	.8 mg./100 ml.	2.59 mg/100 ml.

The above would indicate that the trichloracetic acid used in the analysis had little effect in increasing the conversion of the pyro to the ortho form.

A study now was made of the analysis of blood samples to which known amounts of ortho and pyro phosphates were added. The results of these tests follow:

Amount and Source of P.	Analysis Gm. phosphorus /100 ml.
2 ml. water - 5 ml. of blood	4.5
2 ml. ortho (1 ml. .1 mg.) - 5 ml. blood	6.4
2 ml. pyro (1 ml. .1 mg.) - 5 ml. blood	4.65

It is apparent from the above that there is only a slight increase in the phosphorus value when the pyro phosphate is added to the blood sample. This increase corresponds to that observed when a solution of the pyro phosphate was analyzed directly. It may be stated then that the blood values obtained in the main portion of this study represent ortho phosphate. It also indicates that ingested pyro phosphates are converted to the ortho form before passing into the general circulation.

In order to determine the site of this conversion it would probably be necessary to examine the blood passing from the digestive tract to the liver. In view of the marked phosphatase activity of intestinal tissue it would be reasonable to expect that the change to the ortho form might occur during the process of assimilation.

SUMMARY

1. Tetra sodium pyro phosphate is not quite as well utilized for bone development as tri sodium ortho phosphate.

2. The acid pyro phosphate is utilized to the same extent as the acid ortho phosphate.

3. The pyro phosphates are converted to ortho phosphates before reaching the general circulation.

4. Essentially only ortho phosphates are determined in blood when using the Youngberg procedure.

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TABLE III

Average Bone Ash and Blood Phosphorus Values

Animal No.	Sex	Phosphorus source	Amount P contributed by supplement per 100 gm. of ration	Percent bone ash	Blood P (mg./100ml of blood	Weight (seven weeks)
1w-b	o+	$NaH_2PO_4 \times H_2O$	41 mg.	22.09	2.47	104 gm.
1wes-b	o+	" "	41	20.86	1.72	105
2w-b	o+	" "	41	21.84	---	94
2wes-b	o→	" "	41	19.69	---	98
3w-b	o→	" "	41	19.28	2.02	94
3wes-b	o→	" "	41	23.91	1.81	104
4w-b	o+	" "	41	21.82	---	70
4wes-b	o+	" "	41	25.35	2.27	84
5w-b	o→	$NaH_2PO_4 \times H_2O$	82	33.33	4.28	118
5wes-b	o→	" "	82	32.61	---	88
6w-b	o→	" "	82	30.44	5.1	106
6wes-b	o+	" "	82	31.13	3.95	120
7w-b	o→	" "	82	30.70	---	110
7wes-b	o+	" "	82	30.42	6.10	90
8w-b	o+	" "	82	31.09	3.15	116
8wes-b	o+	" "	82	30.07	5.50	98

o→ --- Male animals
o+ --- Female animals

TABLE III (Continued)

Animal No.	Sex	Phosphorus source	Amount P contributed by supplement per 100 gm. of ration	Percent bone ash	Blood P (gm./100ml. of blood)	Weight (seven weeks)
9w-m	0→	NaH_2PO_4 x H_2O	164 mg.	38.55	4.7	96 gm.
9wes-m	0+	" "	164	37.81	5.05	107
10w-m	0→	" "	164	36.73	4.78	96
10wes-m	0→	" "	164	42.22	4.35	120
11w-m	0+	" "	164	35.87	5.15	120
11wes-m	0+	" "	164	escaped	---	---
12w-m	0→	" "	164	35.70	7.00	98
12wes-m	0→	" "	164	38.38	6.70	108
7w-r	0→	NaH_2PO_4 x H_2O	246	39.94	6.81	120
7wes-r	0→	" "	246	39.87	8.1	111
8w-r	0+	" "	246	44.15	6.4	109
8wes-r	0+	" "	246	40.84	7.1	118
3w-x	0→	" "	246	39.40	8.2	98
3b-x	0+	" "	246	36.80	6.95	120
2b-x	0+	" "	246	39.20	4.05	122

o→ --- Male animals
o+ --- Female animals

TABLE III (Continued)

Animal No.	Sex	Phosphorus source	Amount P contributed by supplement per 100 gm. of ration	Percent bone ash	Blood P (gm./100ml. blood)	Weight (seven weeks)
5w-r	O→	NaH_2PO_4 x H_2O	164 mg.	39.2	5.8	106 gm.
5wes-r	O→	" "	164	38.45	7.2	135
6w-r	O+	" "	164	39.81	6.3	115
6wes-r	O+	" "	164	40.59	4.7	106
4b-x	O+	" "	164	41.2	5.9	124
4w-x-r	O→	" "	164	36.09	7.8	140
5w-x	O+	" "	164	34.7	4.7	114
7w-s	O+	$Na_4P_2O_7$ x $10H_2O$	41	19.40	2.98	92
7b-s	O+	" "	41	18.43	3.20	90
13w-b	O→	" "	41	49.90	3.2	94
13wes-b	O→	" "	41	21.80	3.4	118
14w-b	O+	" "	41	20.60	3.6	98
14wes-b	O→	" "	41	19.90	3.5	105

O→ --- Male animals
O+ --- Female animals

TABLE III (Continued)

Animal No.	Sex	Phosphorus source	Amount P contributed by supplement per 100 gm. of ration	Percent bone ash	Blood P (gm./100ml. blood)	Weight (seven weeks)
2w-s	♂	$Na_3PO_4 \times 12H_2O$	164 mg.	33.84	6.32	130 gm.
2b-s	♂	" "	164	37.80	7.80	118
3w-s	♀	" "	164	43.4	7.98	98
3b-s	♀	" "	164	40.74	7.56	118
4w-s	♂	" "	164	44.21	8.08	120
4wes-b	♂	" "	164	44.51	7.69	112
Ave.		" "	164	40.75	7.57	116
3w-s	♀	$Na_3PO_4 \times 12H_2O$	82	24.73	5.1	102
3b-s	♀	" "	82	28.55	6.2	105
5w-b	♂	" "	82	34.21	6.1	135
5wes-b	♂	" "	82	31.43	---	121
6w-b	♀	" "	82	37.68	5.92	122
6wes-b	♀	" "	82	30.10	6.20	127
Ave.		" "	82	31.43	5.90	119

♂ --- Male animals
♀ --- Female animals

TABLE III (Continued)

Animal No.	Sex	Phosphorus source	Amount P contributed by supplement per 100 gm. of ration	Percent bone ash	Blood P (gm./100ml. of blood)	Weight (seven weeks)
4w-s	♀	$Na_3PO_4 \times 12H_2O$	41 mg.	19.8	3.5	122 gm
4b-s	♀	" "	41	24.2	4.2	98
7w-b	♂	" "	41	26.41	3.7	108
7wes-b	♂	" "	41	22.52	3.4	90
8w-b	♀	" "	41	28.95	4.25	110
8wes-b	♂	" "	41	28.49	3.15	95
Ave.		" "	41	25.06	3.70	104

♂ --- Male animals
♀ --- Female animals

TABLE III (Continued)

Animal No.	Sex	Phosphorus source	Amount P contributed by supplement per 100 gm. of ration	Percent bone ash	Blood P (gm./100ml blood)	Weight in gm (seven weeks)
1w-s	♂	Basal	None	Died	---	80 gm.
1b-[illegible]	♂	Basal	None	Died	---	66
1w-b	♂	Basal	None	22.3 mg.	2.52	92
1b-b	♀	Basal	None	18.7	.62	98
2w-b	♂	Basal	None	16.4	2.75	60
2wes-b	♀	Basal	None	16.5	---	64
13w-m	♀	Basal	None	14.2	2.92	
13wes-m	♀	Basal	None	17.3	2.7	
Ave.		Basal	None	17.55	2.7	77
9w-r	♀	Basal containing only one percent		36.79	5.1	119
9wes-r	♂	calcium carbonate		41.08	3.8	133
10w-r	♂	" "	"	36.33	4.15	130
10wes-r	♀	" "	"	36.90	5.00	127
7w-x	♀	" "	"	38.71	4.98	124
7b-x	♀	" "	"	42.5	6.80	120
Ave.		" "	"	38.72	4.99	124

♂ --- Male animals
♀ --- Female animals

TABLE III (Continued)

Animal No.	Sex	Phosphorus source	Amount P contributed by supplement per 100 gm. of ration	Percent bone ash	Blood P (gm./100ml. of blood	Weight in gm (seven weeks)
5w-s	♂	$Na_4P_2O_7 \times 10H_2O$	164 mg.	28.08	8.2	132 gm.
5b-s	♂	" "	164	36.56	7.4	112
9w-b	♀	" "	164	36.89	---	117
9wes-b	♀	" "	164	33.90	---	116
10w-b	♀	" "	164	37.86	6.7	115
10wes-b	♂	" "	164	30.82	5.94	116
Ave.		" "	164	33.85	7.06	118
3w-s	♀	$Na_4P_2O_7 \times 10H_2O$	82	23.09	3.95	122
3b-s	♂	" "	82	28.21	---	98
11w-b	♂	" "	82	30.82	---	150
11wes-b	♂	" "	82	23.18	5.04	120
12w-b	♀	" "	82	25.50	3.92	111
12wes-b	♂	" "	82	29.90	4.02	125
Ave.		" "	82	25.25	4.23	115

♂ --- Male animals
♀ --- Female animals

TABLE III (Continued)

Animal No.	Sex	Phosphorus source	Amount P contributed by supplement per 100 gm. of ration	Percent bone ash	Blood P (gm./100ml. of blood	Weight in gm (seven weeks)
1b-x	♀	S. A. P. P.	164 mg.	42.90	6.5	114 gm.
1w-x	♀	" "	164	36.7	4.5	138
2w-x	♀	" "	164	40.4	7.3	137
1w-r	♂	" "	164	39.09	4.68	106
1wes-r	♀	" "	164	39.49	5.12	117
2w-r	♂	" "	164	36.64	5.21	107
2wes-r	♂	" "	164	34.00	4.82	92
Ave.		" "	164	38.18	5.45	116
3w-r	♂	S. A. P. P.	246	38.9	6.9	113
3wes-r	♀	" "	246	41.3	7.21	120
4w-r	♂	" "	246	40.28	7.3	102
4wes-r	♂	" "	246	43.84	6.8	137
3w-x	♂	" "	246	37.6	7.9	135
3b-x	♂	" "	246	41.2	6.03	128
2b-x	♀	" "	246	42.8	5.00	137
Ave.	0	" "	246	41.02	6.75	124

♂ --- Male animals
♀ --- Female animals

Lynch

The comparative utilization of ortho and pyro phosphate in bone development of rats.
