A STUDY OF RACHITOGENIC DIETS WITH SPECIAL REFERENCE TO THE VALUE OF LYSINE SUPPLEMENTATION

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This is to certify that the

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A STUDY OF RACHITOGENIC DIETS WITH SPECIAL REFERENCE TO THE VALUE OF LYSINE SUPPLEMENTATION

By

Te-shing Yang

A Thesis

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INTRODUCTION

Although many attempts have been made to determine vitamin D with both physical and chemical methods, none of these methods can as yet compete with the biological in sensitivity. The rat assay method is still commonly used and recognized as standard for the determination of vitamin D intended for use by mammals, including man. This method involves the production of rickets by the use of a suitable diet. In spite of the years of extensive use of rachitogenic diets the problem of obtaining uniform composition and subsequent uniform production of rickets has not yet been solved.

The classical rachitogenic diets, devised by early workers, have long been subjected to study, but relatively little progress has been made in improving them. The variability of the ingredients used in the diet gave rise to different results in different laboratories. The animals given these diets usually have low resistance to disease, and frequently a few die during the period of preparation. Some of the animals do not develop satisfactory rickets because of insufficient growth. The need therefore exists of improving the diet both from the standpoint of assuring greater uniformity and, if possible, of reducing the time needed

to develop a suitable degree of rickets and to test for vitamin D in various products.

The present study was carried out in the hope that a more satisfactory diet could be developed. Special attention was given to the use of lysine supplementation. This is based on the fact that cereal proteins are generally low in lysine and growth on cereal diets can be appreciably improved by the addition of this amino acid. A supply of the DL-lysine monohydrochloride was made available for this study by Dr. Wadell of the Du Pont Company of New Jersey.

HISTORICAL REVIEW

A few years before vitamin D was known to the world, experimental rickets was intensively investigated by a number of workers. Among them McCollum, Simmonds, Shipley, Park, and Pappenheimer are all well known in this field. Starting from the year 1920, a series of papers under the title "Studies on Experimental Rickets" was published. (1, 2, 3, 4, 5, 6, 7). Various phases concerning rickets and the antirachitic substance were thoroughly studied.

McCollum and his coworkers (1) first used rats to produce experimental rickets. They found that certain diets, when fed to young rats, produced various disturbances in growth and development of the skeleton. These diets had in common the production of irregularities in the calcification of the intercellular substance of the proliferative cartilage or absence of lime salt deposition from the matrix of the tissue. At that time they attributed the condition to a deficiency in fat-soluble vitamin A and calcium.

Following the first experiment, McCollum and his coworkers (2) further observed that, with the addition of cod liver oil to the diets, a fresh deposition of lime salt between the cells of the proliferative zone of cartilage could be observed. The deposition of cal-

cium salts was linear, the width of the line apparently depending on the length of time that the animals had been fed cod liver oil.

A year later McCollum and his coworkers (5) suggested the name "line test" for this method of determining the antirachitic activity of a product. The essential point of the test was the ability of a given substance to cause reappearance of a provisional zone of calcification in the epiphyseal cartilage of animals with severe rickets. The reliability of the test depended upon a suitable diet which could produce bones with calcium-free epiphyseal cartilage and metaphyses.

Much work and effort has been put into the development of rachitogenic diets. Hess, McCann and Pappenheimer (8) failed to produce rickets on a diet deficient in vitamin A. After many trials, McCollum and his coworkers (3) in 1921 were able to develop a comparatively satisfactory rickets-producing diet known as McCollum's Diet 3143, which was composed of yellow maize 33%, wheat 33%, gelatin 15%, wheat gluten 15%, sodium chloride 1%, and calcium carbonate 3%. They indicated the diet must have : (1) a specific disproportion in the calcium-phosphorus ratio, the phosphorus being low, the calcium relatively high, and (2) an insufficiency of the antirachitic substance. With this diet McCollum (4, 6) was able in 1922 to demonstrate the exist-

ence of vitamin D.

In a more detailed study of the fat-soluble vitamin, Steenbock and Black (9) found that McCollum's Diet 3143 induced too much variation in the production of rickets, due to an insufficiency of vitamin A and too much protein. They suggested that a diet composed of yellow corn 76%, wheat gluten 20%, calcium carbonate 3%, and sodium chloride 1% would give far more consistent results. It also had the advantage of greater ease of preparation, and reduced cost. Steenbock and Black's Diet 2965 together with McCollum's Diet 3143 were soon adopted by many laboratories for the determination of vitamin D from various sources. Due to their wide application these diets were studied from different angles by many workers.

The first point of attack was the phosphorus content and the calcium-phosphorus ratio. The importance of these elements had been pointed out by McCollum and his coworkers (3, 5) in the early studies. Karelitz and Shohl (10) first stated that the addition of phosphate to a rickets-producing diet would cause healing of rickets. Shohl, Brown, Rose, Chapman, and Saurwein (11, 12) in a series of experiments, varied the amount of calcium carbonate and sodium dihydrogen phosphate which were added to the modified Steenbock and Black's Diet 2965, causing various levels and ratios. With the same ratio of cal-

cium and phosphorus, a diet was shown to become progressively less rickets-producing as the salt level was raised. Within the range used, the greater the ratio at a given level of phosphorus, the more severe would be the degree of rickets. When phosphorus was very low (0.12%) rickets could be obtained at any calcium phosphorus ratio. The production of rickets with a high calcium diet was impossible when the phosphorus content was more than 0.5%. With a still higher calcium-phosphorus ratio, as 12:1, 24:1, or 56:1, a less severe degree of rickets resulted, but such animals would die early without gain or loss in weight. The optimal calcium-phosphorus ratio (13) for the production of rickets probably lay between 4 and 5 to 1.

As for the effect of the acid-base content in a rickets-producing diet, Shohl and his coworkers (14) made a number of observations. By analysis, the bone showed greater ash content with the neutral diet, smaller with the alkaline diets, and least with the acid diets. Later studies by Shohl (15, 16) indicated that the acid diet tended toward the production of more severe rickets, since alkalinity caused a diminished activity on the ionization of calcium. This factor was directly related to the blood alkalinity, but its mechanism is still not clearly understood.

The composition of the yellow corn used in the Steenbock Diet was studied extensively. Holmes and Tripp (17), upon analyzing samples of the Steenbock Diet from different laboratories, obtained variations in ash, calcium, and phosphorus content. Harris and Bunker (18, 19) determined the calcium and phosphorus in forty samples of corn and found considerable variation. Rachitogenic diets made with these varieties of corn had calcium-phosphorus ratios ranging from 3.98:1 to 6.7:1. Such variations are of sufficient magnitude as to produce different degrees of rickets.

Other factors which might have influenced the rachitogenic diet were the variation of protein and vitamin A content and the fineness of grinding. (13, 17)

Modification of the Steenbock Diet 2965 was made by Ma (20) in 1937. Table corn meal was used instead of ordinary ground corn. With the addition of 25% oatmeal and 1% dried yeast, a comparatively satisfactory rachitogenic diet was obtained.

In 1947 Bills (13) gave a very comprehensive review of the determination of vitamin D by the rat assay method. He mentioned that the U.S.P. method still referred to McCollum's Diet 3143 and Steenbock's Diet 2965 as standard rachitogenic diets. Young rats weighing just over 44 grams were put on either one of the rachitogenic diets for

a preparatory period of 21-24 days. Those rats weighing between 60-80 grams were used and continued immediately for a 7-day test period. The results were judged by the "line test".

A more recent study by Francis (21) called attention to the amino acid requirement of the rats. By comparing the amino acid content in the Steenbock's Diet 2965 with the rat's requirement as determined by Rose in 1937, the data indicated that the diet provides ample quantities of all amino acids save lysine. With the addition of 0.5% lysine to the diet, a satisfactory degree of rickets could be produced in sixteen days. As pure lysine was too expensive to be used in experiments, blood fibrin was tried in the place of lysine and found to be unsatisfactory.

The successful practical synthesis of DL-lysine monohydrochloride by the Du Pont Company has renewed interest in the use of this amino acid product not only for improving the growth-promoting properties of rachitogenic diets, but also of practical mixed feeds containing chiefly cereal products.

EXPERIMENTAL PROCEDURES

I. Preparation of the rations:

The rachitogenic diets were prepared on the day that a group of young rats was available for experiment. Usually 1 kg. of each ration was prepared at the beginning of the experiment. Each ingredient was weighed carefully on the triple beam balance. Yellow table meal, which composes the bulk of the ration, was weighed out first and transferred to a mixing pan. The other ingredients were weighed out carefully, transferred to the mixing pan, and mixed thoroughly with the yellow table meal. The portions to be used were immediately transferred to Fisher cups and the rest stored in brown bottles.

II. Determination of calcium and phosphorus:

1. Determination of calcium. Method from A.O.A.C. 1945, Sixth Edition (22).

a. Ingredients other than calcium carbonate and common salt.

Triplicate 5-10 grams of finely ground samples were weighed carefully into a porcelain crucible. These were ashed in a muffle furnace until carbon-free. Then the ash was moistened with 1 ml. of concentrated

nitric acid. This was followed by drying the contents and again carefully igniting in the muffle furnace till white-colored. After the ash was allowed to cool, 5 ml. of hydrochloric acid was added, allowing the acid to rinse the upper portion of the dish. The material was now evaporated to dryness on a steam bath. The residue was dissolved by adding an accurate measure of 2.0 ml. hydrochloric acid. This was heated for five minutes on a steam bath with a watch glass on the dish. Afterwards the watch glass was washed with water and the residue filtered into a 400-ml. beaker and diluted to 150 ml.

Into the solution 10 drops of bromocresol green indicator were added and then sufficient 30% sodium acetate solution to change the PH to 4.8 - 5.0 (blue). Then the beaker was covered with a watch glass and the solution heated to boiling. The calcium was precipitated slowly by adding 3% oxalic acid solution, a drop every 3-5 seconds, until the PH was changed back to 4.4 - 4.6, indicated by the appearance of a distinct green shade. This solution was boiled for 1-2 minutes and allowed to settle overnight or until clear. The supernatant liquid was filtered through a fritted glass crucible and the beaker and precipitates washed with about 50 ml. of ammonium hydroxide (1+50) in small portions, using a wash bottle which would deliver a very

small stream. The crucible together with the precipitate was then transferred back to the original beaker, and 125 ml. water and 5 ml. sulfuric acid were added at a temperature of 80-90° C. with 0.05N potassium permanganate until a slight pink color was obtained. The titration result was corrected with a blank titration and the percentage of calcium in the samples calculated.

b. Calcium carbonate and common salt. Modified method from William and Furman's "Elementary Quantitative Analysis", Third Edition (23).

Triplicate 0.5 grams samples of calcium carbonate (5 grams samples of common salt) were weighed into a 400-ml. beaker. 20 ml. of water and 5 ml. of concentrated hydrochloric acid were added. The beaker was covered with a watch glass and the mixture was then heated until dissolved. The sides of the beaker and watch glass were then rinsed and the total volume of the solution was diluted to 150 ml.

The precipitation and titration were carried out as previously described.

2. Determination of phosphorus. Method according to A.O.A.C. 1945, Sixth Edition (22).

Triplicate 5-10 grams of samples were weighed accurately into 500-ml Kjeldahl flasks. 5 ml. of concentrated

nitric acid and 10 ml. of concentrated sulfuric acid were added to each flask in the hood. 5 grams of potassium nitrate was added at once and the flask was allowed to stand in the hood until the violence of the reaction was over. Then 50-70 mls. concentrated sulfuric acid was added to each flask and the mixture was then digested to the point where the solution had become nearly colorless. After cooling, 150 ml. distilled water was added and the solution boiled for a few minutes. The solution, after cooling, was filtered into a 300-ml. volumetric flask and diluted to volume.

A 50-ml. aliquot was pipetted into a 250-ml. beaker. Into the solution ammonium hydroxide was added in slight excess. A few drops of nitric acid were added to dissolve any precipitate formed. The solution was stirred vigorously and fifteen grams of crystalline ammonium nitrate added, 60 ml. of the molybdate solution was then added and the solution digested at about 65° C. for one hour. The yellow precipitate was filtered and washed with ammonium hydroxide solution (1+9) and then dissolved from the filter with ammonium hydroxide (1+1) and hot water into a beaker to a volume of not more than 100 ml. The solution was then neutralized with hydrochloric acid, using bromothymol blue as the indicator and then cooled. 15 ml. of magnesia mixture was added slowly from a burette

(about one drop per second) and the solution subjected to vigorous stirring. After 15 minutes, 12 ml. of concentrate ammonium hydroxide was added. The solution was allowed to stand until the supernatant liquid was clear. The precipitate was then filtered and washed with ammonium hydroxide solution (1-9) until the washings were practically free from chlorides. The precipitate was then dried and burned at low heat and ignited to constant weight in an electric furnace at 950°-1000° C. The residue was cooled in a desiccator and weighed as $Mg_2P_2O_7$. From this the percentages of phosphorus of the samples were calculated.

III. Care of animals:

Young rats approximately three weeks of age and weighing 45-55 grams were placed on the rachitogenic diet in separate cages. Tap water was given ad libitum. Weights were recorded at the end of each week. At the end of two or three weeks, when most of the animals gained at least 20 grams, they were put on a supplemental diet for from 5-10 days. At the end of the test period the rats were killed by ether vapors. The radii were removed for the line test.

IV. Line test:

The wrist bones, after being preserved in 95% alcohol for at least 24 hours, were split and then immersed for 2 minutes in 2% silver nitrate solution. The bones were then transferred to a small porcelain dish containing distilled water and exposed either to natural or artificial light until a desired darkness developed in the calcified area of the bone. Results were then judged by observing the epiphyseal line of calcification.

DATA

TABLE I

The calcium and phosphorus content of various ingredients.

NAME OF INGREDIENT	% CALCIUM	% PHOSPHORUS
Yellow table meal	0.00561	0.158
Yeast	0.00167	1.125
Oil meal	0.428	0.925
Wheat gluten	0.0922	0.276
Casein	0.647	1.088
Whole wheat	0.0566	0.328
Gem Iodized salt	0.0236	--
Morton's Iodized salt	0.0379	--
Calcium carbonate	39.85	--

EXPERIMENT I

TABLE II

Composition of rachitogenic diets.

Name of Ingredient	No. of Diet					
	1	2	3	4	5	6
Yellow table meal	73%	72%	68%	63%	68%	68%
Wheat gluten	20	20	25	30	20	20
Casein (crude)	--	--	--	--	5	--
Oil meal	--	--	--	--	--	5
Yeast	3	4	3	3	3	3
$CaCO_3$	3	3	3	3	3	3
NaCl	1	1	1	1	1	1
Ca %	1.22	1.22	1.22	1.23	1.25	1.24
P %	0.204	0.214	0.210	0.216	0.251	0.243
Ca:P	5.95	5.70	5.81	5.68	4.99	5.13

TABLE III

Growth response of young rats fed the above rachitogenic diets.

No. of Diet	No. of Rats	Average Initial Weight	Av. Wt. End of 3 weeks	Av. Wt. Gained 3 weeks	Av. Wt. Gained Daily
		grams	grams	grams	grams
1	8	47	73	26	1.2
2	8	49	77	28	1.4
3	7	50	76	26	1.2
4	7	50	83	33	1.5
5	8	46	80	34	1.6
6	7	47	77	20	1.0

Line test:

2 drops of standard cod liver oil containing 5 U.S.P. units given by mouth on the first day of the test period. The rats were continued on the same rachitogenic diets for 10 days.

Results:

All groups gave good responses.

EXPERIMENT II

TABLE IV

Composition of rachitogenic diets.

Name of Ingredient	No. of Diet		
	2	7	8
Yellow table meal	72%	71%	71.5%
Wheat gluten	20	20	20
Yeast	4	4	4
$CaCO_3$	3	3	3
NaCl	1	1	1
Lysine	--	1	0.5
Ca %	1.22	1.22	1.22
P %	0.214	0.211	0.213
Ca:P	5.70	5.73	5.72

TABLE V

Growth response of young rats fed the above rachitogenic diets.

No. of Diet	No. of Rats	Average Initial Weight	Av. Wt. End of 3 weeks	Av. Wt. Gained 3 weeks	Av. Wt. Gained Daily
		grams	grams	grams	grams
2	12	51	76	25	1.2
7	12	50	85	35	1.7
8	12	51	84	33	1.5

Line test:

1 drop of standard cod liver oil (5 U.S.P. units) was given by mouth on the first and sixth day of the test period, the rats being kept on the same rachitogenic diets for 10 days.

Results:

All groups gave very good responses.

EXPERIMENT III

TABLE VI

Composition of rachitogenic diets.

Name of Ingredient	No. of Diet 1	9	10	11	12	13
Yellow table meal	73%	72.5%	68%	67.5%	63%	62.5%
Whole ground wheat	--	--	5	5	10	10
Wheat gluten	20	20	20	20	20	20
Yeast	3	3	3	3	3	3
$CaCO_3$	3	3	3	3	3	3
NaCl	1	1	1	1	1	1
Lysine	--	0.5	--	0.5	--	0.5
Ca %	1.22	1.22	1.22	1.22	1.22	1.22
P %	0.204	0.204	0.203	0.203	0.221	0.221
Ca:P	5.95	5.95	6.00	6.00	5.52	5.52

TABLE VII

Growth response of young rats fed the above rachitogenic diets.

No. of Diet	No. of Rats	Average Initial Weight	Av. Wt. End of 3 weeks	Av. Wt. Gained 3 weeks	Av. Wt. Gained Daily
		grams	grams	grams	grams
1	3	48	76	28	1.4
9	3	53	81	26	1.2
10	4	53	74	21	1.0
11	4	49	82	33	1.5
12	3	46	66	20	1.0
13	4	53	88	35	1.7

Line test:

4 units of vitamin D in 1 cc. corn oil, mixed with 50 grams of each rachitogenic ration, were given to the rats as supplementary diet for 10 days.

Results:

All groups gave good responses. There was a slight difference in the degree of response with increases in the amount of phosphorus present in the rachitogenic diet. Those groups with 0.5% lysine gave slightly better responses than the similar groups without lysine.

EXPERIMENT IV

TABLE VIII

Composition of the rachitogenic diets.

Name of Ingredient	No. of Diet 14
Yellow table meal	62.5%
Wheat gluten	30
$CaCO_3$	3
Yeast	3
NaCl	1
Lysine	0.5
Ca %	1.23
P %	0.215
Ca:P	5.70

TABLE IX

Growth response of young rats fed the above rachitogenic diets.

No. of Diet	No. of Rats	Average Initial Weight	Av. Wt. End of 2 weeks	Av. Wt. Gained 2 weeks	Av. Wt. Gained Daily
		grams	grams	grams	grams
14	24	47	75	28	2.0

Line test:

At the end of two weeks all rats were given supplements of fluid vitamin D in a small cup. The 24 animals were divided into 4 groups:

(1) 5 ml. vitamin D milk containing 2 U.S.P. units,

(2) 7-1/2 ml. " " " 3 " " ,

(3) 10 ml. " " " 4 " " ,

(4) 10 ml. ordinary milk.

The rats were killed at the end of 5 days.

Results:

(1) gave broken lines of healing,

(2) gave fairly good responses,

(3) gave very heavy responses, and most of them tended to calcify downward. The lines were not clear.

(4) gave a negative response.

EXPERIMENT V

TABLE X

Growth response of young rats fed rachitogenic diets No. 4 and No. 14.

No. of Diet	No. of Rats	Average Initial Weight	Av. Wt. End of 2 weeks	Av. Wt. Gained 2 weeks	Av. Wt. Gained Daily
		grams	grams	grams	grams
14	12	45	75	30	2.1
4	12	46	64	18	1.3

Line test:

Rats on Diet No. 14 at the end of 2 weeks were given supplements of 7.5 ml. (3 U.S.P. units) vitamin D milk, whereas rats on Diet No. 4 were given similar supplements at the end of the 5th day.

Results:

Rats on Diet No. 14 gave a mostly negative line test.

Rats on Diet No. 4 gave fairly good responses.

EXPERIMENT VI

TABLE XI

Composition of rachitogenic diets.

Name of Ingredients	No. of Diet	
	15	16
Yellow table meal	52.5%	53%
Wheat gluten	40	40
Yeast	3	3
$CaCO_3$	3	3
NaCl	1	1
Lysine	0.5	--
Ca %	1.24	1.24
P %	0.227	0.228
Ca:P	5.46	5.43

TABLE XII

Growth response of young rats fed the above rachitogenic diets.

No. of Diet	No. of Rats	Average Initial Weight	Av. Wt. End of 2 weeks	Av. Wt. Gained 2 weeks	Av. Wt. Gained Daily
		grams	grams	grams	grams
15	12	45	69	24	1.7
16	10	45	68	23	1.6

Line test:

At the end of 2 weeks all rats were given supplements of 7.5 ml vitamin D milk (3 U.S.P. units) in addition to the rachitogenic diets. All rats were killed at the end of the fifth day.

Results:

All gave negative response.

EXPERIMENT VII

TABLE XIII

Composition of rachitogenic diets.

Name of Ingredient	No. of Diet	
	14	17
Yellow table meal	62.5%	61.5%
Wheat gluten	30	30
Yeast	3	4
$CaCO_3$	3	3
NaCl	1	1
Lysine	0.5	0.5
Ca %	1.23	1.23
P %	0.215	0.225
Ca:P	5.70	5.46

TABLE XIV

Growth response of young rats fed the above rachitogenic diets.

No. of Diet	No. of Rats	Average Initial Weight	Av. Wt. End of 2 weeks	Av. Wt. Gained 2 weeks	Av. Wt. Gained Daily
		grams	grams	grams	grams
14	8	45	66	21	1.5
17	7	48	70	22	1.6

Line test:

At the end of two weeks all rats were given a supplementary diet containing 10 ml. vitamin D milk mixed with 40 grams of the ration. All rats were killed at the end of a week.

Results:

Rats on Diet No. 14 gave slight or negative line tests.

Rats on Diet No. 17 showed broken lines of calcification.

All metaphyses were wide and clear.

EXPERIMENT VIII

Rachitogenic Diet No. 17 was used, with one variation, numbered 17'. Diet No. 17' had the same composition as No. 17, except that ordinary yellow table meal was substituted for finely ground yellow table meal.

TABLE XV

Growth response of young rats fed the above rachitogenic diets.

No. of Diet	No. of Rats	Average Initial Weight	Av. Wt. End of 2 weeks	Av. Wt. Gained 2 weeks	Av. Wt. Gained Daily
		grams	grams	grams	grams
17	6	42	69	27	1.9
17'	6	42	64	22	1.6

Line test:

At the end of two weeks 10 ml. vitamin D milk, incorporated with 40 grams of the ration which did not contain lysine, was given as supplementary diet. The rats were killed at the end of a week.

Results:

All gave very slight responses with just the sign of a beginning of line formation.

EXPERIMENT IX

TABLE XVI

Composition of rachitogenic diets.

Name of Ingredient	No. of Diet			
	17	18	19	20
Yellow table meal	61.5%	61.5%	61%	61%
Wheat gluten	30	30	30	30
Yeast	4	4	4	4
$CaCO_3$	3	3	3	3
NaCl	1	1	1	1
Lysine	0.5	0.5	1.0	1.0
$NaHCO_3$	--	0.2	--	0.4
Ca %	1.23	1.23	1.23	1.23
P %	0.225	0.225	0.224	0.224
Ca:P	5.46	5.46	5.48	5.48

TABLE XVII

Growth response of young rats fed the above rachitogenic diets.

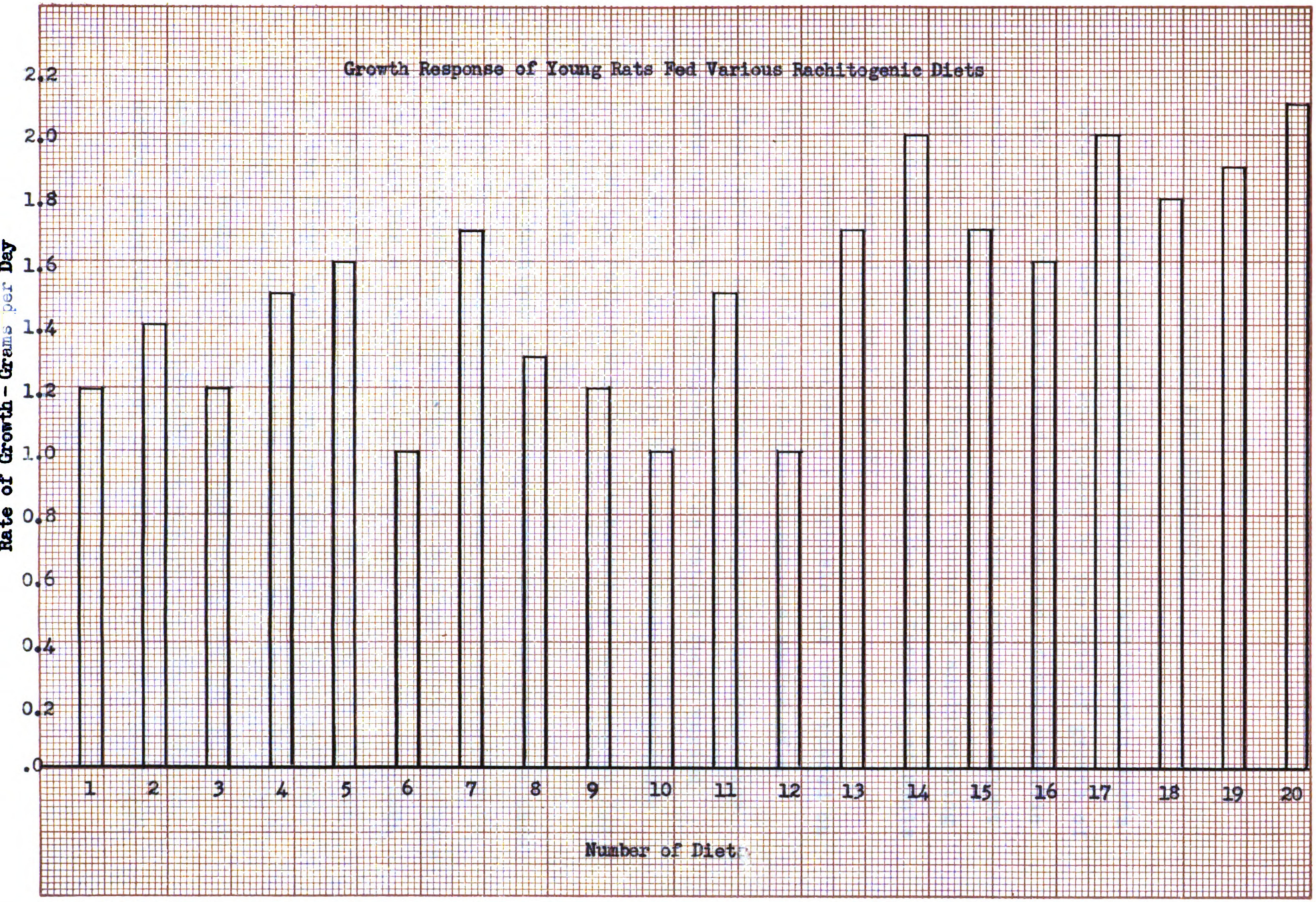
No. of Diet	No. of Rats	Average Initial Weight	Av. Wt. End of 2 weeks	Av. Wt. Gained 2 weeks	Av. Wt. Gained Daily
		grams	grams	grams	grams
17	12	47	75	28	2.0
18	10	46	69	25	1.8
19	12	46	72	26	1.9
20	12	45	75	30	2.1

Line test:

At the end of two weeks all rats were given supplements of 7.5 ml. of vitamin D milk incorporated with 40 grams of the ration. Rats were killed at the end of one more week.

Results:

All rats gave very good responses. More uniform and clearer lines were obtained with the rats receiving Diets No. 18 and No. 20.


Growth Response of Young Rats Fed Various Rachitogenic Diets
Rate of Growth - Grams per Day
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Number of Diet

RESULTS AND DISCUSSION

In this series of studies twenty rachitogenic diets were used. Each diet was judged by the rate of growth of the rats, by the width and the character of the rachitic metaphyses of the radii and the ulnae, and by the extent and nature of the calcification induced by administering a vitamin D supplement. The results of these experiments are presented in tables I-XVII.

Inasmuch as considerable emphasis has been placed on the importance of the calcium-phosphorus ratios of a rachitogenic diet, calcium and phosphorus analyses were made of all the ingredients. The ratios found varied from 4.99 to 6.00. There was no particular correlation between the ratios and the suitability of the rations for determining vitamin D.

The Michigan State College standard rachitogenic diet (Diet No. 2) and five modifications were used for an initial exploratory experiment. The rats were fed the various rations for a three-week preparatory period and a vitamin D supplement during the ten-day test period. At the end of the first trial, all animals gave good line-test responses. The casein in Diet 5 definitely helped the growth of the young rats. This was undoubtedly due to the enrichment of the diet in phosphorus and in the essential amino acids, especially lysine, in which cereals are strikingly

deficient. Increasing the amount of yeast as well as the wheat gluten also showed beneficial effects, whereas oil meal had less influence.

The results of Experiment II show that the rate of growth was appreciably increased with a supplement of 0.5% of DL-lysine monohydrochloride which was a synthetic product supplied by the Du Pont Company. It was especially noticed that growth was more rapidly initiated during the first week. A comparison of the diets with 0.5% and 1% lysine supplementation showed that the higher level did not increase the rate of growth. Some rats on the 1% lysine diet developed inflamed eyelids, but this may have been due to other causes. The responses to vitamin D were good as to the quantity of the calcification. However, a diffused type of calcification occurred which was more noticeable at the 1% level. This type of response is undesirable because it is more difficult to evaluate.

As had been pointed out by Karelitz and Shohl (10), increasing the amount of phosphorus in the diet would decrease the amount of vitamin D required or increase the response to a given amount of vitamin D. In the experiment with the addition of 5 and 10 percent. whole wheat, the result of the line test showed increasing responses with the increased phosphorus supplied by the whole wheat. Lysine supplementation again resulted in an appreciable

increase in the rate of growth.

Further studies were made by the addition of 0.5% lysine to Diet 4, which was the simplest and cheapest to prepare and would support a good growth rate. The test period started at the end of two weeks, since all the rats had gained more than 20 grams. Fairly good results were obtained with 7.5 ml. of vitamin D milk fed at the beginning of a test period of 5 days. By comparing the results with diets 4 and 14, the favorable effect of lysine supplementation is obvious.

A higher level of wheat gluten diet was tried. Results showed that a 40% level was too high. In a direct comparison between the 30% and 40% wheat gluten diets, the former gave much better growth.

On trying a higher level of yeast, better growth was found at the 4% level. This was undoubtedly due to the larger amount of vitamin B complex supplied in the yeast as well as to the phosphorus and protein.

A study was made of the influence of the fineness of grinding of the yellow table meal. The rats showed somewhat better growth on the diet containing the finely ground meal, which was used in subsequent experiments. No particular differences in the line-test response were observed, however.

The last phase of the investigation was a study of

the influence of the hydrochloric acid introduced in the use of lysine monohydrochloride. A comparison was therefore made with a diet supplementation with lysine monohydrochloride only and another containing in addition sodium bicarbonate, equivalent to the hydrochloric acid present in the lysine monohydrochloride. All rats could be used for the test at the end of two weeks. 7.5 ml. of vitamin D milk (3 U.S.P. units) incorporated with 40 grams of the diet gave very good line-test responses in a one-week test period. 0.5% lysine supplementation seemed to give clearer lines than the 1% lysine. Slightly better results were obtained with the rations containing sodium bicarbonate.

In view of the variability of the biological assay for vitamin D, even under careful management it would be desirable to make further comparative studies in the use of lysine to speed growth and the development of rickets. The results obtained indicate that the time needed to prepare the rats for the test could be shortened to two weeks and the test period to one week. This would effect an overall saving of 10 days in the determination of vitamin D and simplify the management of the test. Further work is also needed to determine the cause of the diffused character of the calcification observed in the lysine-fed rats.

SUMMARY AND CONCLUSION

1. Modifications of the Michigan State College standard basal rachitogenic diet were used in the production of rickets in rats. Better growth was obtained by increasing the amount of wheat gluten or yeast or by the addition of casein or oil meal.

2. With the supplementation of 0.5% and 1% synthetic lysine monohydrochloride to the basal diet, much better growth resulted. However, the 1% lysine level did not show any greater effect than the 0.5%.

3. A gain in weight of over twenty grams at the end of two weeks could be produced by adding 0.5% lysine to the modified diet containing 30% wheat gluten.

4. With the addition of an amount of sodium bicarbonate equivalent to the hydrochloric acid present in the lysine monohydrochloride, better results were obtained in the line test.

5. As a result of lysine supplementation, rats can be prepared for a vitamin D assay in two weeks. The test period can be shortened to one week. However, the deposition of calcium salt is somewhat diffused instead of linear, making the evaluation of the response more difficult.

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