# A STUDY OF THE INFLUENCE OF WATER INTAKE ON THE CONDITION OF RATS MAINTAINED ON A MARGINAL DIET WITH AND WITHOUT THE ADDITION OF SODIUM DIHYDROGEN PHOSPHATE

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Priscilla F. Hedan

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[redacted]
Michigan State College
East Lansing, Michigan

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By

Priscilla F. Iledan

An Abstract

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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ABSTRACT

It has been shown in previous experiments that limitation of, or excessive consumption of water in dogs and rats increased phosphorus excretion. This study was an attempt to demonstrate the effect of water consumption on the condition of rats given a marginal diet with and without the addition of sodium dihydrogen phosphate.

Rats were given unlimited amounts of drinking water for the first two weeks, and restricted amounts during the next two weeks. Growth gains and food and/or phosphorus intakes were recorded. Conclusions were obtained by means of analyses of variances, with the amount of drinking water, food and/or phosphorus consumed, and sex as variables.

The results indicated that rats given unlimited amounts of water grew more than those with restricted amounts, where the same kind of diet was employed. The males, on an average, had a greater growth gain than the females. When the same amount of sodium dihydrogen phosphate was added to the different diets, the rats fed diets containing more milk and less rice gained more than those fed less milk and more rice. Addition of sodium dihydrogen phosphate to these experimental diets resulted in a decrease in growth. This decrease was in proportion to the amount of phosphate added.

After the rats had been subjected to a limited water intake for three to twenty-six weeks, they were sacrificed at weekly intervals, and the blood inorganic phosphorus content determined. Results showed that the type of diet fed and the

sex of the experimental animal had no significant effect on the inorganic phosphorus content of the blood. It was,however, influenced by the age of the rat and the length of time when they were given limited amounts of drinking water. Longer periods of restricted water intake resulted in a lower blood phosphorus content. Younger rats showed a higher phosphorus content than the older ones.

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# INTRODUCTION

# INTRODUCTION

Phosphorus was discovered by Brandt in 1669. It is one of the most important inorganic elements involved in animal life. No other one enters into such a diversity of compounds and plays an important part in so many functions. It is a constituent of cell nuclei and all cell structure; it is also prominent in the skeleton, in milk, in glandular tissue and the nervous system. Through the intermediary formation of lecithins, it is concerned with the metabolism of fats. Through the formation of hexosephosphates, of adenylic acid, adenosine diphosphate, and adenosine triphosphate, it plays a primary role in carbohydrate metabolism. There have been some suggestions that it is involved in protein metabolism(1). Phosphates also play an important role in the pH regulation of the organism. These and many more make phosphorus indispensable for life.

Although the compounds of phosphorus participate in numerous important functions of the body it is significant that the literature provides very little information about the influence of water consumption in relation to the intake of phosphorus in the diet.

In 1913 Osborne and Mendel (2) restricted rats to a diet somewhat deficient in phosphorus but unfortunately their only observations were on growth. Since that time there have been numerous investigations of phosphorus in relation to rickets but the principal emphasis has been on

the Ca:P ratio.

The purpose of the present study, therefore, was to study the influence of water intake on the condition of rats maintained on a diet with and without the addition of sodium dihydrogen phosphate.

# HISTORICAL

## HISTORICAL

The necessity of phosphorus for the growth of plants, bacteria, and animals has been known since the very beginning of the biochemical era initiated by Liebig (3). Phosphorus was included in the list of inorganic elements which Liebig stated were necessary for plant growth and as early as 1857 Ville (4) had experimentally proved the requirement of plants for phosphorus in the form of phosphates. However, the early investigators considered phosphates to function chiefly as a structural element of living tissues.

The catalytic and regulatory functions of phosphates in metabolic processes were also investigated during the latter part of the 19th century, but Harden and Young in 1905(5,6) were the first to carry out a more detailed and critical study.

In 1894, Landauer found that deprivation of water increased both nitrogen and phosphorus excretion, and that after reaching a certain limit, the phosphorus elimination returned to normal (7).

Straub (8) studied the effects of thirst in dogs. He found out that phosphorus elimination in the urine during periods of 3-4 days without water intake was increased, though only to a slight extent, about 5-10 %.

In connection with his thorough investigation of the effects of water consumption on metabolism, Hawk (1905)

found out that copious water drinking increased the excretion of phosphorus in the urine, this increase being ascribed to increased cellular activity and the accompanying catabolism of phosphorus-containing compounds. In every instance, the excretion of phosphorus was increased above the normal level, the maximum excretion occurring on the second day of the increased water ingestion. From this evidence, they suggested that either great thirst, or unusual consumption of water, will increase the phosphorus excretion, though further work is required to establish the source of this phosphorus, and therefore, the significance of this increased excretion (9).

During the First World War, Embden et al (10) conducted experiments on German soldiers with acid-phosphate drinks. With the introduction of the phosphate drink, the requirement of drinking water by miners became much smaller. When the use of the phosphate drink was discontinued, a heightened thirst immediately manifested itself. They suggested that the reduced need for water during a period of phosphate supplementation can be attributed to decreased perspiration.

The formulation from natural foodstuffs of a suitable diet deficient in phosphorus presents some difficulties because almost all sources of protein contain relatively large amounts of phosphorus (11).

Schneider and Steenbock (12) fed rats a purified diet very low in phosphorus and lacking in Vitamin D, but adequate in all other nutritional requirements (P,0.04%; Ca, 0.5%).

After 12 to 14 days the rats developed rickets, and within six weeks severe skeletal deformities set in. Growth stopped after 4 to 5 weeks, followed by a decline in weight, and death usually occurred by the end of the sixth week. However, when the low phosphorus-diet was supplemented with adequate Vitamin D, an immediate weight loss occurred in the growing rats, which was not regained for more than three weeks. From calcium and phosphorus balances and tissue analyses the authors (13) concluded that Vitamin D induces the utilization of phosphorus by bone, thereby depriving the soft tissues of their supply of phosphorus, which in turn inhibits growth. These animals survived for as long as twenty weeks, in contrast to those not receiving Vitamin D, and consistently developed urinary calculi.

The effect of phosphorus deficiency on growing rats was studied by Forbes (14) in a 270-day body balance and metabolism experiment conducted with paired-feeding controls. In the first experiment, the phosphorus-deficient and phosphorus-supplemented diets contained 0.13% and 0.31% of phosphorus respectively. The corresponding average P content of their bodies after 70 days was 0.94% and 1.03% respectively, a difference of 10%. There was no difference in the growth or utilization of food energy or protein. In the second experiment the low and high phosphorus diets contained 0.13% and 0.65% of P respectively. After 70 days, the phosphorus content of their bodies was 0.99% and 1.12% respectively, a difference of 13%. Here they observed a slight but significant

depression in the digestability of protein. This was attributed to the effect of $NaH_2PO_4$ in the high phosphorus-diet. There was no effect on the utilization of protein or food energy or growth. The P content of the low-phosphorus diet was as low as could be made and yet sufficient for some growth. When the P content was further decreased, no growth was obtainable.

Day and McCollum (15) prepared a diet containing 0.017% phosphorus but adequate amounts of all other nutrients(Ca,0.4 %). Young rats restricted to this ration grew slowly for 5 to 8 weeks, and then declined in weight and died 2 or 3 weeks later. Extreme rarefaction of the skeleton quickly occurred, accompanied by progressive disability in walking, standing, and breathing. Nitrogen retention was positive for 5 weeks and then became negative simultaneously with the onset of loss of weight. The mobilization of calcium and phosphorus from the bones led to a tremendous loss of calcium and a considerable loss of phosphorus. The greater loss of calcium than of phosphorus can be attributed to the fact that part of the phosphorus mobilized from the bones is taken up by the soft tissues to permit their growth. When the phosphorus was increased without the addition of calcium, or when the calcium was reduced in their diet, rickets did not develop(16).

Sherman and Booker (17) found that with diets of constant phosphorus content (0.42%) increasing the percentage of calcium progressively from 0.1% to 0.50% permitted more rapid calcification of the growing body and earlier maturity

and the appearance of senescence.

Phosphorus must be available in the animal diet not only in adequate amounts but also in the proper balance with calcium. The ratio of calcium to phosphorus in the diet exerts a marked influence upon growth, reproduction, ash content of the bones, and the calcium and inorganic phosphorus content of the blood serum. Shohl and Wolbach (18) concluded that, in the absence of Vitamin D, rickets may be produced with not only high-calcium,low-phosphorus and low-calcium,high-phosphorus diets, but also with low-calcium, low-phosphorus rations. This last group occurs in a zone which the ratios of Ca:P have been called normal, thus demonstrating that rickets may be produced with any ratio of Ca:P in the diet. Bethke,Kick, and Wilder (19) have demonstrated that increasing the Ca:P ratio from 1:1 to 5:1 caused a progressive decrease in growth, bone ash, and the percentage of inorganic phosphorus in the blood serum. Decreasing the Ca:P ratio from 1:1 to 0.25:1 decreased the growth and the percentage of calcium in the blood serum but had only a slight effect upon the bone ash. According to their data, the ratio of Ca:P in the ration is more important in determining the growth and calcification than the actual concentration of the elements in the food. They concluded that the most favorable Ca:P ratio for growth and bone formation lies between 2:1 and 1:1 (P,0.55 to 0.69 ). Cox and Imboden (20) found that successful reproduction and lactation in the rat were dependent upon both the actual level and the Ca:P ratio of the diet. The best results were obtained on a diet containing 0.49% each of calcium and phosphorus,

corresponding to a daily intake of about 42 mg. of each element.

Hubbell, Mendel, and Wakeman (21) devised a new salt mixture for use in experimental diets. During a selected period of very rapid growth (from 60 to 200 gram body weight at a rate in excess of 5 grams per day ) on a diet containing this mixture, adequate calcification occurred with an average daily intake of 50 mg. calcium and 35 mg. of phosphorus.

In growing animals the amount of phosphate in the diet (required for bone formation, etc.) is larger than in the mature animal where only sufficient phosphate must be taken up for that excreted.

This can be fully explained in the study of the normal phosphorus content for each sex of rats at various stages of growth and development by Sherman and Quinn (22). Their results showed an average of 0.34% phosphorus in the body at birth to 0.49 % at 15 days; 0.53 -0.56% at 23 days; 0.57-0.65% at 61 days; 0.62-0.63% at 3 months; 0.65-0.69% at 4 months; 0.70-0.75% in adult life. After 15 days of life, the total weight of phosphorus was higher in males than in the females, due to their greater average body weights only. The females that had not borne young showed a higher percentage of phosphorus than males of the same age, heredity, and dietary history. A diet high in beryllium and strontium hindered phosphorus absorption, and like calcium, gave rise to a form of rickets.

Absorption of phosphorus is enhanced by an acid reaction within the intestines. Cohn and Greenberg (23) have recently used radioactive phosphorus in absorption studies. They showed

that the absorption of phosphates by rachitic rats was increased but little by Vitamin D, but the inorganic phosphorus uptake in the bone increased 25 to 50%. They suggested that Vitamin D may act to aid the conversion of organic to inorganic phosphorus. Alkalies, as a rule, decreased phosphorus absorption.

The effect of fat is not clearly established. Some believe that fatty acids combine with calcium and thus favor phosphorus absorption, whereas others have stated that the use of high-fat diets causes an excessive loss of both calcium and phosphorus in the feces.

The deficiency of phosphorus in the food only gradually changes the relative phosphorus content in the organs, and only in the case of poor growth does it become noticeable, especially in the bones (24).

Excessive excretion of phosphates has been reported in certain bone diseases like osteomalacia (25), diffuse periostosis and rickets, in the early stages of pulmonary tuberculosis, in acute yellow atrophy of the liver, in disorders associated with marked breakdown of nerve tissue, and after sleep caused by administration of chloral hydrates or bromides. It is also increased after copious water drinking, as was previously mentioned.

A decrease in the excretion of phosphates is noted in acute infectious diseases accompanied by fever, in pregnancy during the period of fetal bone formation, in diabetes after insulin administration, and in kidney diseases due to failure

of elimination.

The form in which phosphorus must be taken in the body has been the subject of considerable speculation, and ideas which we now know to be erroneous have been widely held.

Of the various classes of phosphorus compounds present in food, Sherman, et al (11) found out that organic combinations appeared to have greater nutritive value than the inorganic forms, and he gave this as a reason for the varying amounts of phosphorus reported necessary for the maintenance of equilibrium in man.

Gregersen (26) found out that rats fed a diet of edestin, lard, sugar, cellulose, and salts, with $NaH_2PO_4$ as the sole source of phosphorus, were able to maintain equilibrium over considerable periods of time, or even exhibited a positive balance. From this he concluded that the organism can build inorganic phosphate into organic phosphate.

According to present-day concepts, the form of phosphorus in the diet is of little importance, since in any case, as a result of the digestive processes, phosphorus is largely absorbed in the inorganic form. This is not surprising when we reflect that in all the complex tissue constituents such as phospholipids, nucleoproteins, and probably phosphoproteins also, the phosphorus is present as phosphoric acid esters. It is to be expected that the organism would be able to build up those esters when provided with a source of inorganic phosphorus.

It is generally agreed that inorganic salts of phosphorus

are readily utilized by the animal when included in the normal ration. However, Eddy, Muller, and Hoft (27) have shown that the phosphorus of phytin, in contrast to inorganic phosphorus, is unable to protect rats receiving a high-calcium, low-phosphorus diet from rickets. Bruce and Callow (28) have confirmed this finding and have shown that treatment of the phytin-containing material with 1% HCl renders the phosphorus available for the cure of rickets, presumably because of the hydrolysis of the inositol phosphoric acid. Inasmuch as a large part of the phosphorus of grains and seeds is in the form of phytin, these findings are of importance in considering the phosphorus content of the ration. Probably all of the phosphorus of the leaves and stems of plants is available to the organism for these contain very little, if any, phytin.

It may be noted that the requirement of phosphorus varies, depending on the diet, the age and sex of the animal, and other factors.

In view of the importance of phosphorus to the health and well-being of all animals, as well as human beings, it was of interest to study the influence of water consumption on the condition of rats maintained on a marginal diet with and without the addition of sodium dihydrogen phosphate.

## EXPERIMENTAL

### Animals and Diets:

Male and female albino rats of a strain developed in the Michigan State College Chemistry Department were mostly 21 to 25 days old when selected for these experiments. A few rats, between two to three months of age, were also set up to observe differences in growth, if any, due to age. White rats were selected as the experimental animals for this study because of their omnivorous food habits, the close resemblance of the chemical processes of human and rat metabolism, the convenient size of the rat for investigation of this character, and the fact that many features of the nutrition and life histories of the rat have been extensively studied by numerous investigators. A total of 112 rats were studied. In each set, 16 animals were divided into groups of 4, with each group having an equal number of males and females weighing mostly from 45 to 60 grams. The initial weights, total weight gains, and total dietary intake are listed in Table II. Table III shows their average weight gains, dietary intake, and phosphorus intake per day. A percentage comparison of growth gain to food and/or phosphorus consumption is included in Table IV.

Different experimental diets were prepared as shown in Table I. One-half % and 1 % $NaH_2PO_4$ were added to Diets 2 and 3 respectively using Diet I as the control. Diets 5 and 6 were made up of 0.5% and 1% $NaH_2PO_4$ respectively, with Diet 4

as the control. The different components were finely ground and thoroughly mixed so as to ensure uniform composition of the diets. These were stored in dark bottles at room temperature.

During the first two weeks of each experiment, the animals were given unlimited food and drinking water. After that period, they had free access to food, but their drinking water was limited to 15 milliliters per day (20 ml. per day for the older rats).

A careful record of their weekly food consumption was made. The food intake was calculated from the difference between the total amount supplied and the tare weight corresponding to the food spilled. The animals were kept in individual, raised cages, and the room temperature was maintained between 75 and 78°F.

After two weeks of limited water supply, and weekly intervals thereafter, the animals were sacrificed, and the blood phosphorus determined.

Analytical Method for Blood Inorganic Phosphorus:

After sacrificing the animal using ether, blood was quickly removed and placed in beakers having a thin dry film of anticoagulant.

The method used for analysis was essentially that of Fiske and Subbarow (29). It is based on the fact that when trichlor-acetic acid is added to blood, the proteins are precipitated; treated with an acid molybdate solution, this acid-

TABLE I

COMPOSITION OF EXPERIMENTAL DIETS

Components (per 100 grams of food)

| Diet No. | Rice (G.) | Linseed Oil Meal (G.) | Alfalfa (G.) | Whole Milk, Dwd. (G.) | NaCl (G.) | $NaH_2PO_4$ (G.) | P per 100 G. Food * (G.) |
|---|---|---|---|---|---|---|---|
| 1 | 79.0 | 10 | 5 | 5 | 1 | -- | 0.213 |
| 2 | 78.5 | 10 | 5 | 5 | 1 | 0.5 | 0.330 |
| 3 | 78.0 | 10 | 5 | 5 | 1 | 1.0 | 0.493 |
| 4 | 59.0 | 10 | 5 | 25 | 1 | -- | 0.323 |
| 5 | 58.5 | 10 | 5 | 25 | 1 | 0.5 | 0.433 |
| 6 | 58.0 | 10 | 5 | 25 | 1 | 1.0 | 0.540 |

* Control Diets I and I/ were analysed; the rest were calculated.

TABLE II

WEIGHT GAINS AND FOOD CONSUMPTION

| Diet No. | Unlimited Drinking Water: Initial Weight (G.) | Final Weight (G.) | Total Weight Gain (G.) | Total Food Intake (G.) | Limited Drinking Water: Initial Weight (G.) | Final Weight (G.) | Total Weight Gain (G.) | Total Food Intake (G.) |
|---|---|---|---|---|---|---|---|---|
| | | | | YOUNG RATS: | | | | |
| 1 | 79 | 120 | 41 | 1 2 | 127 | 1 2 | 35 | 1 3 |
| 1 | 55 | 102 | 47 | 174 | 112 | 15 | 44 | 211 |
| 1 | 55 | 103 | 51 | 190 | 103 | 14 | 40 | 202 |
| 1 | 55 | 97 | 42 | 152 | 97 | 118 | 21 | 124 |
| 1 | 43 | 3 | 32 | 118 | 30 | 105 | 25 | 115 |
| 1 | 50 | 93 | 43 | 154 | 93 | 138 | 45 | 219 |
| 2 | 52 | 101 | 49 | 139 | 101 | 141 | 40 | 17 |
| 2 | 57 | 105 | 48 | 152 | 105 | 12 | 21 | 123 |
| 2 | 48 | 106 | 58 | 110 | 106 | 12 | 20 | 147 |
| 2 | 52 | 8 | 3 | 15 | 3 | 13 | 45 | 220 |
| 3 | 76 | 12 | 50 | 193 | 13 | 13 | 25 | 1 9 |
| 3 | 59 | 98 | 39 | 171 | 104 | 134 | 30 | 1 9 |
| 4 | 100 | 1 9 | 69 | 203 | 173 | 19 | 25 | 172 |
| 4 | 53 | 12 | 70 | 1 | 139 | 17 | 37 | 1 2 |
| 4 | 51 | 115 | 64 | 146 | 115 | 1 3 | 48 | 177 |
| 4 | 54 | 110 | 5 | 12 | 110 | 13 | 23 | 130 |
| 4 | 48 | 10 | 58 | 110 | 10 | 126 | 20 | 147 |
| 4 | 47 | 94 | 47 | 10 | 94 | 142 | 48 | 200 |
| 5 | 51 | 129 | 7 | 1 9 | 129 | 157 | 28 | 1 5 |
| 5 | 55 | 102 | 47 | 113 | 102 | 129 | 27 | 122 |
| 5 | 4 | 94 | 48 | 30 | 94 | 117 | 23 | 122 |
| 5 | 48 | 104 | 56 | 132 | 104 | 159 | 25 | 20 |
| 6 | 92 | 143 | 51 | 1 1 | 150 | 1 3 | 3 | 171 |
| 6 | 54 | 103 | 54 | 14 | 120 | 152 | 32 | 1 1 |
| | | | | OLDER RATS: | | | | |
| 1 | 20 | 223 | 22 | 205 | 23 | 262 | 24 | 214 |
| 3 | 206 | 226 | 20 | 224 | 23 | 243 | 10 | 1 4 |
| 4 | 211 | 24 | 3 | 1 3 | 241 | 273 | 32 | 1 5 |
| 6 | 225 | 251 | 26 | 1 7 | 255 | 27 | 23 | 1 7 |

TABLE III

AVERAGE WEIGHT GAINS AND AVERAGE DIETARY INTAKE

| Diet No. | Growth Gain Per Day Male (g.) | Growth Gain Per Day Female (g.) | Food Consumed Per Day Male (g.) | Food Consumed Per Day Female (g.) | P Intake Per Day * Male (mg.) | P Intake Per Day * Female (mg.) | Number of Rats |
|---|---|---|---|---|---|---|---|
| I-A. Unlimited Water Supply (Young Rats): | | | | | | | |
| 1 | 2.99 | 2.80 | 11.00 | 11.10 | 29.50 | 29.16 | 24 |
| 2 | 3.07 | 2.70 | 11.00 | 10.20 | 41.35 | 38.67 | 16 |
| 3 | 3.95 | 2.70 | 14.65 | 11.65 | 72.23 | 57.44 | 8 |
| 4 | 4.37 | 3.87 | 10.70 | 9.67 | 34.59 | 31.22 | 24 |
| 5 | 4.25 | 3.63 | 8.95 | 8.63 | 38.93 | 37.52 | 16 |
| 6 | 4.45 | 2.70 | 12.65 | 10.70 | 70.42 | 58.63 | 8 |
| I-B. Limited Water Supply: | | | | | | | |
| 1 | 2.43 | 1.93 | 12.82 | 11.47 | 34.30 | 30.73 | |
| 2 | 2.08 | 2.08 | 10.81 | 10.82 | 41.09 | 38.24 | |
| 3 | 2.75 | 2.65 | 13.40 | 12.80 | 65.86 | 63.11 | |
| 4 | 2.72 | 2.12 | 11.92 | 11.00 | 37.49 | 36.03 | |
| 5 | 2.38 | 1.78 | 10.11 | 9.63 | 43.99 | 42.09 | |
| 6 | 2.95 | 1.80 | 13.10 | 11.25 | 71.79 | 61.65 | |
| II-A. Unlimited Drinking Water (Older Rats): | | | | | | | |
| 1 | 2.60 | 1.90 | 17.10 | 14.20 | 19.03 | 38.05 | 4 |
| 3 | 1.70 | 1.40 | 15.30 | 15.60 | 75.43 | 73.91 | 4 |
| 4 | 1.80 | 1.70 | 14.20 | 13.80 | 45.87 | 44.57 | 4 |
| 6 | 1.10 | 1.40 | 15.30 | 13.50 | 83.34 | 73.98 | 4 |
| II-B. Limited Drinking Water: | | | | | | | |
| 1 | 1.80 | 1.50 | 18.40 | 13.60 | 39.21 | 36.45 | |
| 3 | 0.70 | 1.60 | 16.50 | 13.80 | 81.34 | 67.13 | |
| 4 | 2.00 | 1.70 | 17.30 | 13.60 | 55.18 | 43.93 | |
| 6 | 2.00 | 1.70 | 18.00 | 14.30 | 98.64 | 78.36 | |

* Calculated from actual food consumption

TABLE IV

COMPARISON OF GROWTH GAIN

TO FOOD AND/OR PHOSPHORUS CONSUMPTION

| Diet No. | Growth Gain Per Day (g.) / Food Consumed Per Day (g.) | | Growth Gain Per Day (g.) / P Intake Per Day (mg.) | |
|---|---|---|---|---|
| | Males | Females | Males | Females |
| I-A. Unlimited Drinking Water (for 9 Young Rats): | | | | |
| 1 | 0.27 | 0.25 | 0.101 | 0.096 |
| 2 | 0.28 | 0.27 | 0.074 | 0.070 |
| 3 | 0.27 | 0.23 | 0.055 | 0.047 |
| 4 | 0.46 | 0.47 | 0.128 | 0.129 |
| 5 | 0.49 | 0.43 | 0.112 | 0.093 |
| 6 | 0.36 | 0.26 | 0.065 | 0.043 |
| I-B. Limited Drinking Water: | | | | |
| 1 | 0.19 | 0.17 | 0.072 | 0.064 |
| 2 | 0.19 | 0.21 | 0.051 | 0.055 |
| 3 | 0.21 | 0.21 | 0.042 | 0.041 |
| 4 | 0.23 | 0.20 | 0.071 | 0.062 |
| 5 | 0.24 | 0.19 | 0.055 | 0.043 |
| 6 | 0.22 | 0.16 | 0.041 | 0.029 |
| II-A. Unlimited Drinking Water (for 10 Older Rats): | | | | |
| 1 | 0.15 | 0.13 | 0.137 | 0.050 |
| 3 | 0.11 | 0.09 | 0.024 | 0.013 |
| 4 | 0.13 | 0.12 | 0.039 | 0.038 |
| 6 | 0.07 | 0.10 | 0.013 | 0.019 |
| II-B. Limited Drinking Water: | | | | |
| 1 | 0.10 | 0.11 | 0.046 | 0.041 |
| 3 | 0.04 | 0.11 | 0.009 | 0.024 |
| 4 | 0.11 | 0.12 | 0.036 | 0.039 |
| 6 | 0.11 | 0.12 | 0.020 | 0.022 |

free filtrate forms phosphomolybdic acid with any phosphate present. The phosphomolybdic acid is reduced by the addition of 1,2,4-aminonaphtholsulfonic acid reagent, to produce a blue color whose intensity is proportional to the amount of phosphate present.

Inasmuch as inorganic phosphate is slowly liberated from phosphorus compounds on standing (30,31), the blood phosphorus was anlyzed almost immediately after it was drawn.

The amount of anticoagulant used affected the color tremendously. Normal color development was obtained by using about 2-3 milligrams of lithium or potassium oxalate per milliliter of blood. Excessive use of anticoagulant resulted in a diminished color intensity, sometimes to the extent that no color was produced at all (Table V).

The color intensities of the solutions were measured exactly five minutes after the addition of the aminonaphtholsulfonic acid reagent, using the Hellige "Chromatron" photoelectric colorimeter. There was little change in readings at the end of 10, 15 and 20 minutes.

The concentration of inorganic phosphorus in the aliquot was calculated using the density values of the blood sample and the standard phosphate solution; the milligrams of inorganic phosphorus per 100 ml. of blood is equal to

$$\frac{\text{density of sample}}{\text{density of standard}} \times 0.04 \times 100.$$

Analytical Method for Inorganic Phosphorus in Food Samples:

For the determination of inorganic phosphorus in the diet,

TABLE V

ANTICOAGULANT AND COLOR DEVELOPMENT

For lithium oxalate ( 2-3 mg. per ml. of blood recommended)(32)

| Amount used: | Color intensity: |
|---|---|
| 2 mg./ml. of blood | Normal color development |
| 3.5 mg. ml. of blood | " " " |
| 8.0 " " " " | Diminished color development |
| 11.0 " " " " | Practically no color development |
| 30.5 " " " " | " " " " |

For sodium citrate ( 5 mg. per ml. of blood recommended)(32)

| Amount used: | Color intensity: |
|---|---|
| 5 mg./ml. of blood | Normal color development |
| 8 " " " " | " " " |
| 16 " " " " | " " " |
| 25 " " " " | Very slight color " |
| 40 " " " " | Practically no color development |

a weighed sample was placed in a porcelain dish, charred slowly over a free flame, and finally burned at a dull red heat in a muffle furnace to a white ash. The ash was dissolved with hydrochloric acid, the solution filtered, and made up to 200 ml. A 50 ml. aliquot of this solution was then withdrawn, diluted and heated on a steam bath for several hours until the solution had evaporated to approximately 40 ml. This solution was then employed for the determination of phosphorus, by the method of double precipitation, first as ammonium phosphomolybdate and then as ammonium-magnesium-phosphate, with final weighing as magnesium pyrophosphate, all essentially as described in the methods of analysis of the Association of the Official Agricultural Chemists (33).

## RESULTS

The figures found in Table II are the averages of a group of four young rats, each containing two males and two females. Sixteen rats between 2 to 3 months of age were set up for Diets 1,3,4,and 5 at the earlier part of the experiment. No rats of the latter age group were fed Diets 2 and 5.

A comparison was made between the growth rate of the rats and the various factors involved in the experiment. The variables considered were the amount of drinking water supplied, the type of diet employed, and/or the amount of phosphorus consumed from said diet, and the sex of the experimental animal. The conclusions were obtained by means of analyses of variances, based on the figures found in Table VI.

A two factor analysis was made on the blood inorganic phosphorus content of rats which received a limited amount of drinking water. The type of diet used (for source of phosphorus) and the length of time when drinking water was restricted were the two variables considered. The results found in Table IX were obtained from rats which were 21 to 25 days old at the outset of the experiment. Those included in Table XI involves both the young male rats (21 to 25 days old) as well as the older ones (3 months).

Explanation of symbols used:

D - type of diet

$D_1$ Control #1

$D_2$ " " plus 0.5% $NaH_2PO_4$

$D_3$ " " " 1% "

$D_4$ Control #2

$D_5$ " " plus 0.5% $NaH_2PO_4$

$D_6$ " " " 1% "

W - amount of drinking water

$W_1$ Unlimited water

$W_2$ Limited water

S - sex of the experimental animal

$S_1$ Male rat

$S_2$ Female rat

T - time period when rats were given limited amounts of drinking water

$T_1$ 3 weeks

$T_2$ 4½ "

$T_3$ 5 "

$T_4$ 5 "

$T_5$ 7 "

$T_6$ 8 "

$T_7$ 10 "

$T_8$ 13 "

$T_9$ 24 "

$T_{10}$ 26 "

TABLE VI

A. Ratios of Growth Gain Divided by Food Consumed

| | | $D_1$ | $D_2$ | $D_3$ | $D_4$ | $D_5$ | $D_6$ |
|---|---|---|---|---|---|---|---|
| $W_1$ | $S_1$ | 0.27 | 0.28 | 0.27 | 0.46 | 0.49 | 0.36 |
| | $S_2$ | 0.25 | 0.27 | 0.23 | 0.47 | 0.43 | 0.26 |
| $W_2$ | $S_1$ | 0.19 | 0.19 | 0.21 | 0.23 | 0.24 | 0.22 |
| | $S_2$ | 0.17 | 0.21 | 0.21 | 0.20 | 0.19 | 0.16 |

B. Ratios of Growth Gain Divided by Phosphorus Consumed

| | | $D_1$ | $D_2$ | $D_3$ | $D_4$ | $D_5$ | $D_6$ |
|---|---|---|---|---|---|---|---|
| $W_1$ | $S_1$ | 0.101 | 0.074 | 0.055 | 0.123 | 0.112 | 0.065 |
| | $S_2$ | 0.096 | 0.070 | 0.047 | 0.129 | 0.093 | 0.043 |
| $W_2$ | $S_1$ | 0.072 | 0.051 | 0.042 | 0.071 | 0.055 | 0.041 |
| | $S_2$ | 0.064 | 0.055 | 0.041 | 0.062 | 0.043 | 0.029 |

TABLE VII

ANALYSIS OF VARIANCE OF THE RATIOS OF GROWTH GAIN DIVIDED BY FOOD CONSUMED

| Source of Variance | Degree of Freedom | Mean Square $F_{(34)}$ |
|---|---|---|
| Total | 23 | 220,203*** |
| D | 5 | 11,543*** |
| S | 1 | 6,370** |
| W | 1 | 109,435*** |
| D X W | 5 | 8,127*** |
| D X S | 5 | 915 |
| Error | 6 | 232 |
| **At $W=W_1$:** | | |
| Total | 11 | 104,827** |
| D | 5 | 19,321** |
| S | 1 | 4,524 |
| Error | 5 | 740 |
| **At $W=W_2$:** | | |
| Total | 11 | 5,392 |
| D | 5 | 1,776 |
| S | 1 | 2,030 |
| Error | 5 | 2,036 |

NOTE:

* = Fisher ratio significant at the 5% level
** = " " " " " 1% "
*** = " " " " "0.1% "

TABLE VII-A

Overall Sums and Averages for Variables
Growth Gain Divided by Food Consumed

| Variables | X | $\bar{X}$ | Variables | X | $\bar{X}$ |
|---|---|---|---|---|---|
| $D_1$ | 0.33 | 0.22 | | | |
| $D_2$ | 0.95 | 0.24 | $S_1$ | 3.41 | 0.23 |
| $D_3$ | 0.92 | 0.23 | $S_2$ | 3.01 | 0.25 |
| $D_4$ | 1.36 | 0.34 | | | |
| $D_5$ | 1.35 | 0.34 | $W_1$ | 4.02 | 0.34 |
| $D_6$ | 1.00 | 0.25 | $W_2$ | 2.40 | 0.20 |
| | <u>At W=$W_1$:</u> | | | <u>At W=$W_2$:</u> | |
| $D_1$ | 0.52 | 0.26 | $D_1$ | 0.36 | 0.13 |
| $D_2$ | 0.55 | 0.23 | $D_2$ | 0.40 | 0.20 |
| $D_3$ | 0.50 | 0.25 | $D_3$ | 0.42 | 0.21 |
| $D_4$ | 0.93 | 0.47 | $D_4$ | 0.43 | 0.22 |
| $D_5$ | 0.92 | 0.46 | $D_5$ | 0.43 | 0.22 |
| $D_6$ | 0.62 | 0.31 | $D_6$ | 0.38 | 0.19 |
| $S_1$ | 2.13 | 0.36 | $S_1$ | 1.28 | 0.21 |
| $S_2$ | 1.91 | 0.32 | $S_2$ | 1.14 | 0.19 |

CHART I
FIRST ORDER INTERACTION D X W
AT LEVELS $W = W_1$ & $W_2$

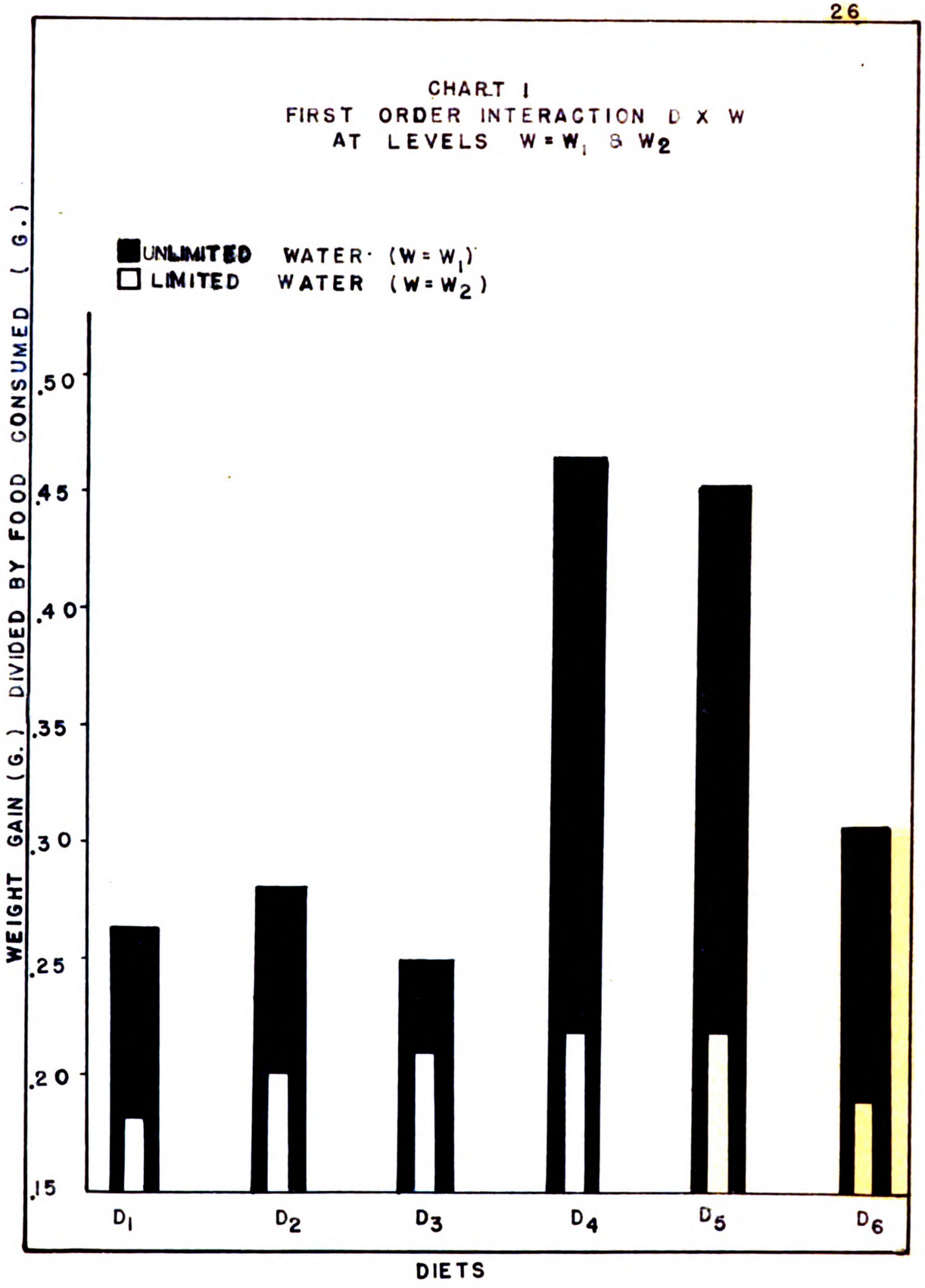

TABLE VIII

ANALYSIS OF VARIANCE OF THE RATIOS OF GROWTH GAIN DIVIDED BY PHOSPHORUS CONSUMED

| Source of Variance | Degree of Freedom | Mean Square $_F$ |
|---|---|---|
| Total | 23 | 13,067*** |
| D | 5 | 1,731*** |
| W | 1 | 6,590*** |
| S | 1 | 315** |
| D X W | 5 | 456*** |
| D X S | 5 | 32 |
| Error | 6 | 11 |
| **AT $W_1$:** | | |
| Total | 11 | 9,529** |
| D | 5 | 1,826** |
| S | 1 | 197 |
| Error | 5 | 41 |
| **AT $W_2$:** | | |
| Total | 11 | 1,891* |
| D | 5 | 1,721 |
| S | 1 | 122 |
| Error | 5 | 105 |

TABLE VIII-A

Overall Sums and Averages for Variables
Growth Gain Divided by Phosphorus Consumed

| Variable | X | $\bar{X}$ | Variable | X | $\bar{X}$ |
|---|---|---|---|---|---|
| $D_1$ | 0.33 | 0.03 | | | |
| $D_2$ | 0.25 | 0.07 | $W_1$ | 1.02 | 0.09 |
| $D_3$ | 0.13 | 0.05 | $W_2$ | 0.63 | 0.05 |
| $D_4$ | 0.39 | 0.10 | | | |
| $D_5$ | 0.31 | 0.03 | $S_1$ | 0.87 | 0.07 |
| $D_6$ | 0.13 | 0.05 | $S_2$ | 0.73 | 0.07 |
| | <u>At W=W1:</u> | | | <u>At W=W2:</u> | |
| $D_1$ | 0.20 | 0.10 | $D_1$ | 0.14 | 0.07 |
| $D_2$ | 0.14 | 0.07 | $D_2$ | 0.11 | 0.06 |
| $D_3$ | 0.10 | 0.05 | $D_3$ | 0.03 | 0.04 |
| $D_4$ | 0.26 | 0.13 | $D_4$ | 0.13 | 0.07 |
| $D_5$ | 0.21 | 0.11 | $D_5$ | 0.10 | 0.05 |
| $D_6$ | 0.11 | 0.06 | $D_6$ | 0.07 | 0.04 |
| $S_1$ | 0.54 | 0.09 | $S_1$ | 0.33 | 0.06 |
| $S_2$ | 0.49 | 0.03 | $S_2$ | 0.29 | 0.05 |

CHART 2

FIRST ORDER INTERACTION D X W

AT LEVELS $W = W_1$ & $W_2$

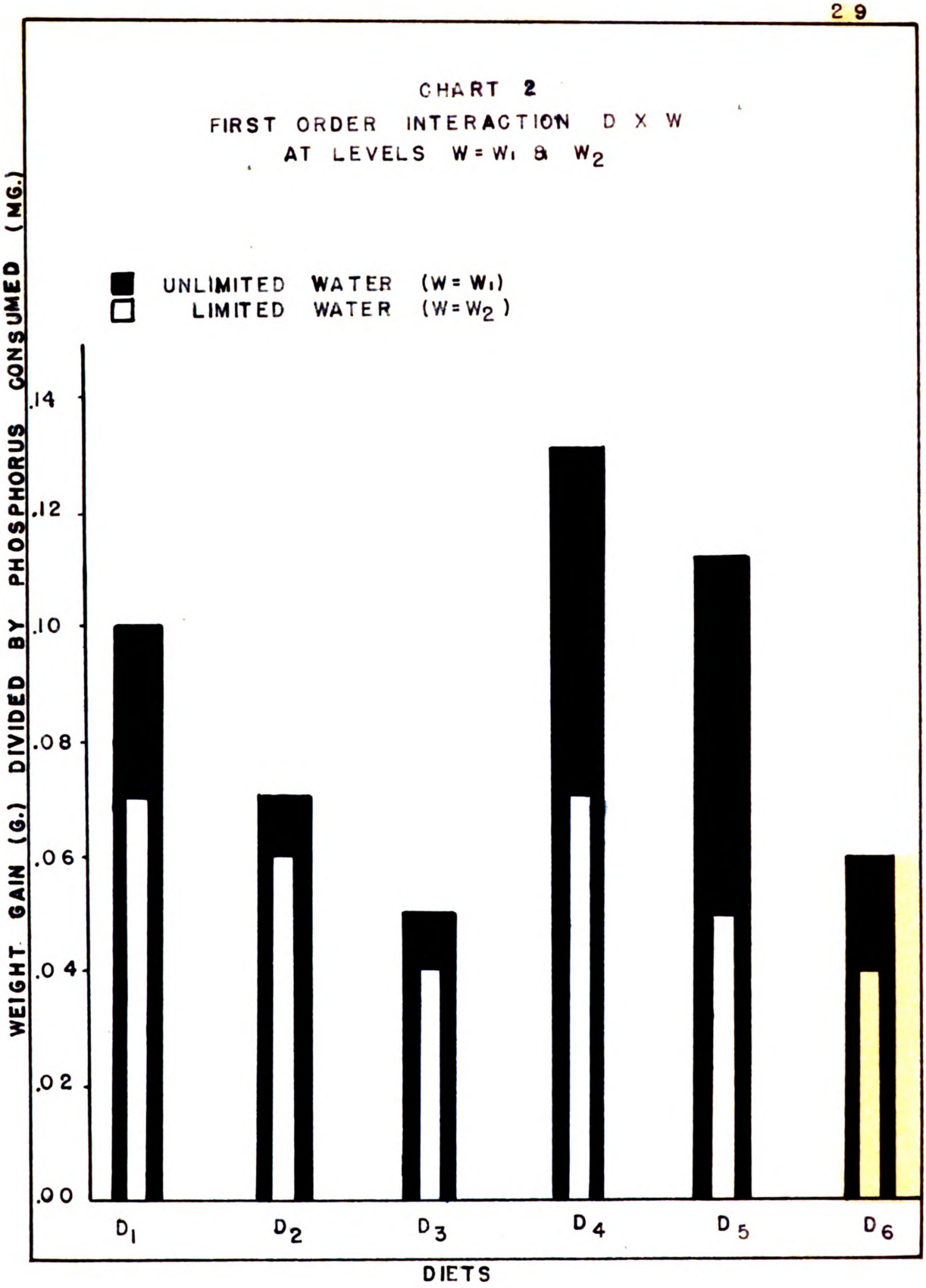

TABLE IX

BLOOD PHOSPHORUS CONTENT OF YOUNG RATS GIVEN LIMITED AMOUNTS OF DRINKING WATER

| Rat No. | Diet No. | Sex | No. of Weeks on Limited Water | mg.P/100 ml. of Blood |
|---|---|---|---|---|
| 98 | 1 | Female | 3 | 6.96 |
| 102 | 2 | " | 3 | 7.36 |
| 106 | 4 | " | 3 | 6.84 |
| 110 | 5 | " | 3 | 5.40 |
| 81 | 1 | Male | 4½ | 5.36 |
| 85 | 2 | " | 4½ | 6.52 |
| 89 | 4 | " | 4½ | 6.92 |
| 93 | 5 | " | 4½ | 6.12 |
| 82 | 1 | Female | 5 | 7.04 |
| 86 | 2 | " | 5 | 6.40 |
| 90 | 4 | " | 5 | 6.20 |
| 94 | 5 | " | 5 | 7.88 |
| 99 | 1 | Male | 5 | 6.76 |
| 103 | 2 | " | 5 | 5.40 |
| 107 | 4 | " | 5 | 5.88 |
| 111 | 5 | " | 5 | 7.24 |
| 83 | 1 | Male | 7 | 7.04 |
| 87 | 2 | " | 7 | 6.56 |
| 91 | 4 | " | 7 | 7.32 |
| 95 | 5 | " | 7 | 7.92 |
| 100 | 1 | Female | 8 | 5.04 |
| 104 | 2 | " | 8 | 5.36 |
| 108 | 4 | " | 8 | 5.56 |
| 112 | 5 | " | 8 | 6.16 |
| 84 | 1 | Female | 10 | 4.64 |
| 88 | 2 | " | 10 | 5.10 |
| 92 | 4 | " | 10 | 5.88 |
| 96 | 5 | " | 10 | 6.10 |
| 49 | 1 | Male | 13 | 5.68 |
| 53 | 2 | " | 13 | 4.12 |
| 57 | 4 | " | 13 | 5.32 |
| 61 | 5 | " | 13 | 5.80 |

TABLE X

ANALYSIS OF VARIANCE OF THE BLOOD PHOSPHORUS CONTENT OF YOUNG RATS

| Source of Variance | Degree of Freedom | Sum of Squares | Mean Squares | F |
|---|---|---|---|---|
| Total | 31 | 257,166 | | |
| D | 3 | 21,750 | 7,250 | |
| T | 7 | 137,662 | 19,660 | ** |
| Error | 21 | 77,755 | 3,703 | |

Overall Sums and Averages of Variables

| Variable | X | $\bar{X}$ | Variable | X | $\bar{X}$ |
|---|---|---|---|---|---|
| $T_1$ | 26.56 | 6.64 | $D_1$ | 48.52 | 6.07 |
| $T_2$ | 24.92 | 6.23 | $D_2$ | 47.32 | 5.92 |
| $T_3$ | 27.52 | 6.88 | $D_4$ | 49.72 | 6.22 |
| $T_4$ | 25.08 | 6.20 | $D_5$ | 52.92 | 6.22 |
| $T_5$ | 28.84 | 7.21 | | | |
| $T_6$ | 22.12 | 5.53 | $S_1$ | 24.89 | 6.22 |
| $T_7$ | 22.72 | 5.68 | $S_2$ | 24.72 | 6.18 |
| $T_8$ | 20.72 | 5.18 | | | |

TABLE XI

BLOOD PHOSPHORUS CONTENT OF YOUNG AND OLDER RATS GIVEN LIMITED AMOUNTS OF DRINKING WATER

| Rat No. | Diet No. | Age of Rats at Beginning of Exp. | No. of Weeks on Lim. $H_2O$ Supply | mg.P/100 ml.Blood |
|---|---|---|---|---|
| 33 | 1 | 3 months | 24 | 4.29 |
| 37 | 3 | " " | 24 | 4.63 |
| 41 | 4 | " " | 24 | 4.20 |
| 45 | 6 | " " | 24 | 4.56 |
| 17 | 1 | 21-25 days | 26 | 4.72 |
| 21 | 3 | " " " | 26 | 5.20 |
| 25 | 4 | " " " | 26 | 4.43 |
| 29 | 6 | " " " | 26 | 5.52 |

TABLE XII

ANALYSIS OF VARIANCE OF THE BLOOD PHOSPHORUS CONTENT OF YOUNG AND OLDER MALE RATS

| Source of Variance | Degree of Freedom | Mean Square |
|---|---|---|
| Total | 7 | 14,090 |
| D | 3 | 2,271 |
| T | 1 | 5,995* |
| Error | 3 | 427 |

Overall Sums and Averages For Variables

| Variables | X | $\bar{X}$ |
|---|---|---|
| $D_1$ | 9.01 | 4.25 |
| $D_3$ | 9.33 | 4.94 |
| $D_4$ | 8.68 | 4.34 |
| $D_5$ | 10.03 | 5.04 |
| $T_9$ | 17.73 | 4.43 |
| $T_{10}$ | 19.92 | 4.93 |

# DISCUSSION

# DISCUSSION

Within the limits of this experiment, the amount of drinking water supplied, and the type of diet used and/or the amount of phosphorus from the different diets have a very marked influence on the growth of albino rats. The factor with the least significance is the sex.

## Food Intake Versus Growth Rate:

As indicated in Table VII, the three variables involved exerted a marked influence on the growth rate of the rats. Of these factors, the amount of drinking water supplied had the greatest effect, and the sex of the animal, the least effect.

With an unlimited water intake, growth rate of the rats was 0.34 compared to 0.20 at a limited water intake.

Rats given Diets 4 and 5 gave the highest gain weight (0.34 and 0.34 respectively), followed by those that had Diet 6 (0.25). Those given Diets 1,2,and 3 had the lowest average growth rate (0.22, 0.24, and 0.23). This is as expected inasmuch as Diets 4, 5, and 6 contained more milk.

The sex of the animal also had an effect on growth. Males had a greater weight gain (0.28) as compared to the females (0.25).

Of the interactions indicated by this three factor analysis, the only significant one was D X W. This indicates that the effect of water supply on growth is dependent on the

type of diet employed, and vice versa. A clear picture of this interaction can be seen on Chart I. On the other hand, the effect of sex is independent of any other main effect.

The growth rate of rats kept on an unlimited water supply is greatly affected by the type of diet employed. Rats fed Diets 4 and 5 gave an appreciably higher weight gain (0.47 and 0.46 ) than those fed Diet 6 (0.31). Diets 1, 2, and 3 gave the lowest growth (0.26, 0.23 and 0.25 respectively). The sex of the animal did not affect the rate of growth.

With a limited water intake, the type of diet employed and sex did not affect the rate of growth.

Phosphorus Intake versus Growth Rate:

From Table VIII, it is apparent that the amount of drinking water given, the amount of phosphorus consumed from the different diets, and the sex of the experimental animal are the important factors affecting growth. The interaction involving the amount of phosphorus consumed and the water supplied is also significant, which implies that those two factors are dependent upon each other where growth is concerned. A graphical interpretation of this interaction is shown in Chart 2.

The most significant factor is the amount of water supplied. When drinking water was unlimited, growth rate was 0.09 compared to 0.05 when water was limited.

The phosphorus intake is also of importance. Based on the same amount of phosphorus, rats fed Diet 4 gave the greatest growth (0.10), followed by those given Diets 1 and 5 (0.08

and 0.08). Growth rate was lowest for those given Diets 2,3, and 6 (0.07, 0.05 and 0.05 respectively).

As shown in Chart 2, an increase in phosphorus content by the addition of sodium dihydrogen phosphate resulted in a slower rate of growth. This was true whether drinking water was limited or not. The deleterious effect on growth was greater on Diets 4,5, and 6 than on Diets 1,2, and 3.

When drinking water was unlimited the type of diet from which phosphorus was acquired was of significance. The sex of the rat did not affect the rate of growth.

The sex characteristic of the experimental animal was not important when water supply was limited. Phosphorus consumed from the different diets, however, was significant. Rats given Diets 1 and 4 had the greatest growth (0.07 and 0.07) followed by those which had Diets 2, 3, 5, and 6 (0.06, 0.04, 0.05 and 0.04 respectively).

Blood Inorganic Phosphorus Content:

As seen in the two factor analysis of variance on Table X, the type of diet and sex of the experimental animal did not affect the blood inorganic phosphorus content. The only factor influencing the phosphorus content was the time that the rats had been subjected to a limited amount of water. Blood phosphorus content was about the same during a period of 3 to 8 weeks of limited amounts of drinking water ($T_1$=6.64mg./100 ml. of blood, $T_2$=6.22, $T_3$=6.88, $T_4$=6.27, $T_5$=7.21); it

decreased when water was restricted for a longer period of time ($T_6$=5.53, $T_7$=5.68, $T_8$=5.18).

When young and older rats were given a limited water supply, the blood phosphorus content was not affected by the type of diet used (Table XI). However, it was greatly influenced by the length of time when the rats were given restricted amounts of drinking water. After 26 weeks of limited water intake, young rats had a blood inorganic phosphorus content of 4.98 mg./100 ml. of blood. Older rats had 4.43 mg. of phosphorus after 23 weeks of restricted amounts of drinking water.

# SUMMARY

## SUMMARY

On the basis of weight gain per food and/or phosphorus intake, the following conclusions were reached:

1. Rate of growth was influenced by the type of diet fed, the amount of drinking water supplied, and the sex of the experimental animal.
2. Rats kept on an unlimited water supply had a greater growth rate than those with restricted amounts.
3. The males, on an average, had a greater growth gain than the females.
4. When the same amount of sodium dihydrogen phosphate was added to the different diets, the rats fed diets containing more milk and less rice gained more than those fed diets containing less milk and more rice.
5. When sodium dihydrogen phosphate was added to these diets, growth decreased in proportion to the amount of phosphates added.
6. With an unlimited water supply, the type of diet had a marked effect on the growth rate of young rats.
7. Sex did not affect growth when water supply was unlimited.
8. On a limited water intake, the amount of phosphorus consumed exerted an influence on the rate of growth.
9. Male rats kept on a limited water supply had almost the same growth gain as compared to the female ones.

On the basis of blood inorganic phosphorus content, the following conclusions were reached:

1. The type of diet fed to the rats did not affect the inorganic phosphorus content of the blood.
2. Sex of the experimental animal had no significant effect on the blood phosphorus content.
3. The level of blood inorganic phosphorus was influenced by the length of time to which the rats were subjected to a limited amount of drinking water. Longer periods of restricted water intake resulted in a smaller amount of phosphorus in the blood.
4. The age of the rat was important in relation to blood inorganic phosphorus content. Younger rats had a higher phosphorus content than the older ones.

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Department of Chemistry
Michigan State College
East Lansing, Michigan