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INFLUENCE ON THE GROWTH OF
RATS OF VARIOUS MODIFICATIONS
OF THE SHERMAN-CHASE DIET
FOR A VITAMIN B₁ ASSAY

Thesis for the Degree of M. S.
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INFLUENCE ON THE GROWTH OF RATS OF VARIOUS MODIFICATIONS
OF THE SHERMAN-CHASE DIET FOR A VITAMIN B₁ ASSAY

by

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Introduction

The Sherman-Chase (1) diet for assaying food for vitamin B₁ (thiamin) by the rat growth method has been used since 1931, when the antineuritic vitamin and a growth factor contained in yeast were thought to be the only vitamin B factors necessary for rat growth. More recently, studies on several other vitamin B components have been published. Some of these factors may be low or lacking in the basal diet, which depends on autoclaved yeast for the B factors other than thiamin. With the availability of many of these vitamin B factors in crystalline form it appeared possible to test whether the autoclaved yeast in the Sherman-Chase diet was supplying all these B factors in amounts necessary for normal growth. Therefore this study was undertaken to determine which of the vitamin B factors, in addition to the basal diet containing autoclaved yeast and thiamin chloride, would induce better growth under our laboratory conditions, and if sulfite treated yeast replacing the autoclaved yeast would give a more complete basal diet.

Review of Literature

The use of the growth of standardized young rats as a means of detecting the presence of vitamin B₁ in foods was early suggested, and a method was suggested by Sherman and Spohn (2) in 1923. The basal diet developed by them was generally used up to the time when it was demonstrated that for normal growth the rat required in addition to vitamin B₁ a heat stable fraction remaining in autoclaved yeast. The Sherman-Spohn diet was then modified by Chase (Sherman-Chase diet) (1) so that vitamin B₁ was considered then to be the first if not the only growth limiting factor. The use of the preliminary depletion period was included as a precautionary measure.

During the last few years, it has been definitely established that the heat stable fraction of yeast (called B₂) is a mixture of a number of vitamins. Riboflavin, nicotinic acid, vitamin B₆ (pyridoxine), Factor W, pantothenic acid, "filtrate factor" are substances which have been studied in their relation to vitamin B₂. Some of these factors are essential for growth of the rat, others may or may not be essential. The question has been raised whether or not all these fractions are supplied in autoclaved yeast in amounts necessary for optimum growth, and whether differences in laboratory manipulation would influence the amounts in the autoclaved yeast.

Following the demonstration by Goldberger and Lillie (3) and by Smith and Hendrich (4) of the independent existence of what is now recognized as the complex B₂ apart from the antineuritic vitamin B₁, the hypothesis was widely accepted that vitamin B₂ would prevent or

cure rat dermatitis, canine black tongue, and human pellegra, which were consequently regarded as strictly analogous diseases. In 1930, Roscoe (5) produced experimental evidence to the effect that vitamin B₂ was a complex, composed of two dietary essentials--vitamin B₂ and factor Y (B₆), necessary for growth of rats. They found Factor Y present in autoclaved yeast. Sure, Smith and Kik's (6) experimental work also showed there were two vitamin B₂ factors. Then in 1933 Gyorgy, Kuhn, and Wagner-Jauregg (7) showed that riboflavin (lactoflavin and ovoflavin) possessed intense vitamin activity. Very soon Gyorgy (8) demonstrated that riboflavin was essential for rat growth but distinct from the rat dermatitis preventive factor (B₆). Chick, Copping and Edgar (9) confirmed Gyorgy's work, and Lepkovsky and Jukes (10) also proved it necessary for growth in rats and chicks. Thus it is definitely known that riboflavin is necessary for normal growth of the rat. McHenry and Gavin (11), also Birch (12) used 10 gamma of riboflavin in their vitamin B free diet, though they did not include autoclaved yeast, for satisfactory growth.

When Goldberger and Lillie (3), working on human pellegra, found a factor (P-P factor) present in yeast which cured the disease, they thought rat dermatitis was caused by the lack of the same factor, though it has since been proven by Gyorgy (8) however that rat dermatitis is due to a lack of vitamin B₆. So it was not until Elvehjem, Maddedn, Strong, and Woolley (13) isolated nicotinic acid and found that it was specific for black tongue in dogs, (analogous to human pellegra), that the exact difference between the two types of dermatitis was recognized. Olesen, Bird, Elvehjem and Hart (14) thought nicotinic acid might be

essential for growth of rats. However Dann and Subbarow (15), also Birch (16) added 1 milligram of nicotinic acid to the B₁ test diet and found no increase in gain in weight, thus concluded that nicotinic acid was not essential for growth of the rat. Dinick expresses the theory "that undoubtedly nicotinic acid must play a role in rat metabolism since it forms a part of the enzyme system involving hydrogen transfer in the metabolism of carbohydrates. Either, like vitamin C it can be synthesized by the rat or its stores are so well conserved as to render exhaustion from the rat body tissues difficult or impossible."

As indicated above vitamin B₅ prevents and cures a nutritional dermatitis in rats (Gyorgy (8)), and Lepkovsky (13) showed that the addition of it to the diet caused a resumption of growth. It is probably identical with the substances designated Factor Y by Chick and Copping (19) with vitamin H of Hogan (20) and of Booher (21), and with Factor I crystallized by Lepkovsky (22).*

A recent paper by Dimmich and Schaffler (24) showed that rats receiving crystalline Factor I (B₆) utilized food better than those on the deficient diet. They found that 10 gamma of crystalline vitamin B₆ satisfied the rat requirement for normal growth.

In 1935 Elvehjem, Koehn, and Closon (25) presented evidence of the existence in liver of a thermolabile substance necessary for the growth of rats, which they believed to be different from any member of the B complex previously described, and they proposed the name Factor

*In 1939 Gyorgy (23) and Eckhardt suggested that in accordance with the chemical nature of vitamin B₆ the term pyridoxine to be an appropriate name. This year (1940) at the American Institute of Nutrition it was recommended by the Committee on vitamin Nomenclature that vitamin B₆ be called "Pyridoxine."

W for this substance. It is an alcohol-ether precipitate from water extract of hog liver, and is heat labile. Kline, Tolle, and Nelson (26) believed that autoclaved yeast, as the sole source of vitamin B complex other than vitamin B₁ will be low or devoid of Factor W as it is destroyed in the heating process. These workers demonstrated that the addition of liver extract to a vitamin free diet plus thiamin, synthetic riboflavin, carotene, nicotinic acid, choline increased the growth. No report has been made of the crystallization of Factor W. Therefore, the extracts or the various preparations from liver may contain other factors than Factor W.

Williams, Lyman, Goodyear, Truesdall and Holaday (27) announced the discovery of a growth determinant of universal biological occurrence which was named "pantothenic acid." It is itself unstable but can be concentrated from liver extract or rice bran extract. Williams (28) and Hitching and Subbarow (29) suggested that pantothenic acid is responsible for part of the growth promoting activity of liver extract for rats. Oleson, Woolley and Elvehjem (30) found that rats needed Factor W as well as pantothenic acid for rat growth in addition to the crystalline factors (thiamin, riboflavin, vitamin B₆) which were supplied in the basal diet. Jukes (31) showed that the two filtrate factors--pantothenic acid by Williams (32,33) et. al., and the chick dermatitis factor by Woolley, Waisman, and Elvehjem (34) are probably identical. Therefore there are indications that both pantothenic acid and Factor W are necessary for normal growth though the quantities necessary are not known.

In a basal diet used for assay of foods for vitamin B₁, all these

necessary factors should be included in adequate amounts. In the Sherman-Chase diet, autoclaved yeast is the recognized source of them. Coward, Burn, Ling, and Morgan (35) found that with increased amounts of autoclaved yeast from 3 percent to 20 percent of the basal diet the resulting growth would be greater with the same amount of vitamin B₁ added. This showed the interdependence of the vitamins and that the estimation of the amount of the vitamin B₁ in a food being tested would vary with the supply of the vitamin B₂ factors. Some other method of yeast preparation may be used and has been suggested. The lability of thiamin in neutral medium is well exemplified by the ease of cleavage by sulfite at pH 5 to 6. (Williams, Waterman, Kenztesy, Buchman (36)) into two inactive substances. Kline, Tolle, and Nelson (37) used the above observations as the bases for treating yeast. Their diet was the same as that used by Sherman-Chase except the yeast was treated with sulfite. The animals grew as well as those receiving autoclaved yeast. Booker and Hartzler (38) found that they had the same results in vitamin B₁ assaying of foods when the diet contained sulfite treated yeast as when it contained autoclaved yeast, and that the choice between sulfite-treated yeast and autoclaved yeast could be decided on the basis of convenience of the experiment. Similarly Smith and Cimmich (39) recommended the use of rice bran concentrates treated with sulfur dioxide as an addition to the Sherman-Chase diet as it provided members of the B complex which are often not supplied by the autoclaved yeast.

It is evident from the work reported that the Sherman-Chase diet with the addition of autoclaved yeast is probably low in Factor W and pantothenic acid and the addition of Factor W preparation to the diet

would supply these factors. The stability of vitamin B₆ in yeast to autoclaving is questionable and the addition of the vitamin to the diet may induce better growth.

Experimental Procedure

In this experiment, 14 litters of white rats of known heridity were used as experimental animals. Rats at the age of 25 to 28 days and of a weight of approximately 45 to 55 grams were placed in individual cages with raised screen bottoms of 5/16 inch mesh, which were cleaned twice weekly. They were fed the vitamin B₁ deficient diet for about 2 weeks to deplete the body stores of the vitamin. Distilled water was allowed ad libitum. After one week, harnesses (Page (40)) were used to prevent coprophagy, as consumption of feces has long been known to interfere with a reliable vitamin B₁ assay ((41) Steenbock et. al.). There is evidence that vitamin B₁ is formed in the cecum, probably as the result of bacterial action on the food residue there. Guerrant and Ditcher (42) found that the vitamin B₁ due to refection is not absorbed from the lower intestinal tract but the rat may obtain benefit from it by the simple expedient of consuming his feces. The use of harnesses tends to prevent this.

The consumption of the basal diet was controlled during the entire test so that the rats within each litter were allowed a uniform amount of the basal diet thus containing the same amount of the B factors of autoclaved yeast. The food was weighed twice weekly and each rat received the amount of food for the next half week that the rat with the lowest food consumption had eaten in the previous period. If all the rats in the litter had eaten all the food allowed it would be increased by 5 grams in the next period. This amount of food was divided over the half week consumption period.

After the first week, the rats were weighed every other day and sometimes each day toward the end of the second week. The weight of the rats usually decreased after harnessing but would increase again by the fourth day. When the weight of all the rats in a litter went down the second time or remained stationary, that litter was considered to have been depleted in vitamin B₁. The depleted rats were then placed on supplement, weighed weekly, and the food consumption recorded bi-weekly. The harnesses were removed at the time of weighing to allow the animals to clean themselves, and were not replaced for several hours.

When a litter of rats were depleted of their stores of vitamin B₁, 6 animals from that litter were placed in each of the 6 test groups, making a litter representative in each group. The number of male and female rats and mean weight in each group were distributed equally.

The six groups were fed as follows:

1. Basal diet plus 4 gamma thiamin chloride (control group).
2. Basal diet plus 4 gamma thiamin chloride plus 1 mgm. nicotinic acid.
3. Basal diet plus 4 gamma thiamin chloride plus 10 gamma ribo-flavin.
4. Basal diet plus 4 gamma thiamin chloride plus 10 gamma vitamin B₆.
5. Basal diet plus 4 gamma thiamin chloride plus 0.7 gm. Factor W per week.
6. "Sulfite yeast" diet plus 4 gamma thiamin chloride.

The supplements were continued for the 4 weeks test period. With the exception of the Factor W preparation, the supplements were fed daily

including Sunday by a calibrated pipette directly into the mouth of the experimental animals. Because the animals objected to the bitterness of some of the vitamin solutions, all solutions were made up double strength and each day's portion was diluted with a 10 percent sugar solution. The rats ate with avidity the 0.35 grams of Factor W preparation which was weighed on a sensitive torsion balance, and fed twice a week in a small container. All supplements with the exception of Factor W preparation, were made up in stock solutions and kept in the refrigerator, and from the stock solutions the exact dilutions were made up each week.

The solution of crystalline thiamin chloride was prepared as recommended by the International Committee on Vitamin Standardization of the League of Nations (43). The stock solution was prepared by dissolving 20 milligrams of thiamin chloride (U.S.P. vitamin B₁ reference standard) in 100 ml. of a 25 percent aqueous ethyl alcohol to which was added HCl to make the solution approximately N/500 acid. Then, 8 ml. of this stock solution were diluted to 50 ml. with distilled water. The rats were fed 1/4 ml. of the final solution plus 1/4 ml. of the sugar solution which equalled 4 gamma of thiamin.

The stock solution of crystalline riboflavin (Merck and Co.) was prepared by dissolving 20 milligrams of the riboflavin in 100 ml. N/500 HCl. The solution was kept in a brown bottle in the refrigerator to prevent decomposition by light. Each week 10 ml. of the stock solution was diluted to 50 ml. with distilled water. Thus 1/4 ml. of the final solution plus 1/4 ml. of the sugar solution equalled 10 gamma riboflavin the daily dosage for the rats.

For the nicotinic acid solution, 200 milligrams of pure crystals (Merck and Co.) were dissolved in 100 ml. water. The rats were fed $1/2$ of the stock solution plus $1/2$ ml. of the sugar solution which equalled 1 milligram daily.

To make the stock solution of crystalline vitamin B_5 (pyridoxine) (Merck and Co.), 20 milligrams were dissolved in 100 ml. of distilled water and kept in a dark bottle. Each week 20 ml. of the stock solution was diluted to 50 ml. with distilled water. Thus, $1/4$ ml. of the final solution plus $1/4$ ml. of the sugar solution equalled 10 gamma of the vitamin B_5 .

The Factor W preparation, supplied through the courtesy of the Wilson Laboratories, "is a rather successful source of Factor W and pantothenic acid, and will supply the various members of the B complex with the exception of thiamin chloride.* The animals received 0.7 grams of this preparation each week, which is about equal to 5 percent of the basal diet as recommended by Elvehjem.*

The Sherman-Chase vitamin B_{12} --deficient diet used had the following composition:

Vitamin B free casein	13.7
Salt mixture (Hawk-Oser '31)	4.5
Filtered butterfat	3.0
Cod liver oil	2.0
Autoclaved yeast or Sulfited yeast	15.0
Cornstarch	53.0

*Private communication with Professor C. A. Elvehjem and Dr. David Klein.

The casein was freed of vitamin B₁ by cold extraction with 60 percent ethyl alcohol (by weight), according to the method as described by Spohn and Sherman (2), 300 grams of coarse casein was treated with 1½ liters of 60 percent alcohol, and the whole was shaken for 1 hour, and then allowed to stand for 5½ hours. The mixture was filtered with suction and thoroughly washed with 750 ml. of 60 percent alcohol. The casein was returned to the bottle, another liter of 60 percent alcohol added, and it was again shaken for 1/2 hour. After standing for 12 hours, it was filtered, washed with 750 ml. of 60 percent alcohol, and finally with 1/2 liter of 90 percent (by weight) to facilitate air drying. After drying it was reground.

Hawk and Cser's (44) modification of the Osborn and Mendel salt mixture was used instead of the original formula.

This mixture was composed of:

Ca citrate. 4 HCH		309.67 parts
Ca(H ₂ PO ₄) ₂ .HCH		113.25
K ₂ HPO ₄		213.72
KCl		125.29
NaCl		77.41
CaCO ₃		63.90
MgCO ₃		33.43
MgSO ₄ (anhydrous)		33.50
Fe citrate 1½ HCH	94.13)	
NaF	3.63)	
MnSO ₄	1.17)	13.80
K ₂ Al ₂ (SO ₄) ₄ .24 HCH	0.67)	
KI	0.30)	
		1000.00 parts.

The weighed quantities of the salts were thoroughly mixed and ground in a ball mill. Owing to the slightly higher content of water of crystallization 4.5 percent of this mixture was used in place of 4 percent of the original Osborn-Mendel salt mixture.

The autoclaved yeast was prepared (1) from baker's yeast which had been air dried and ground. A smooth paste was made of the ground baker's yeast and 0.1 M NaOH solution (using for each 100 grams of yeast 125 ml. N/10 NaOH). The mixture was then heated for 6 hours at 15 pounds pressure in a pressure cooker. The resulting yeast was neutralized with an equivalent amount of standard HCl. It was dried in an oven at 60° C. and reground.

In the preparation of the sulfite treated yeast (37), to each 50 grams of dried ground baker's yeast, in a 500 ml. wide mouth bottle was added 400 ml. of 0.1 percent sodium sulfite. Sulfur dioxide was bubbled through until a pH of 4.0 was reached. The bottle was stoppered and kept in the dark at room temperature (25° C) for 5 days. The contents of the bottle was then dried on purified casein at a temperature not exceeding 65° C. At the end of the depletion period this sulfite treated yeast was substituted for the autoclaved yeast in the diet of group 6.

The commercial butter was melted, decanted to remove the milk which separated out and finally filtered in a warming cabinet. Patch's cod liver oil and Argo's cornstarch were used.

To obtain information on whether or not there was any significant difference between gain in weight of the animals on the various supplements an analysis of variance was made according to Snedecor (45). It

was possible to use an analysis of variance because of the perfect litter control--each animal being classified according to the two independent criteria (supplements and litters). In this way the mean variation of all 36 animals could be used instead of the variation within one group. This value of variation is better, being the mean of a larger number and thus a truer mean. Also, all 6 groups could be investigated simultaneously, and this is a distinct gain in time. The chief source of variation was difference between litters, the other source was between supplement groups. The remaining variation may be observed in the irregularity of the response of the individual animals to the supplements fed. This last is ascribed to the experimental error. The problem was, then, to make a valid estimation of the experimental error and to determine by means of it whether there were significant differences between the 6 supplements and between litters. This is the "F" value ($F = \frac{\text{variance}}{\text{error}}$). This F value read from "Student's" table gives an indication whether there is a significant difference between the means. To test which of the groups were significantly different the value of "t" was determined between the thiamin control group and each of the other groups by the formula $t = \frac{\text{difference between means}}{\sqrt{(\text{error variance}/N).2}}$. These values were also looked up in the same table for probability of significances.

By way of summary, all the test groups were controlled so that:

1. Each group had one animal from each litter.
2. Each group had an equal number of males and females.
3. Each group had the same mean weight at the beginning of the test period.
4. Each animal received 4 gamma thiamin chloride each day including

Sunday.

5. The food consumption was controlled within litters so the average food consumption of each group would be about equal.

6. The basal diet was the same for all groups except group 6 which received sulfite treated yeast in place of autoclaved yeast in the basal diet.

7. The only variation was then the addition of the various vitamin B supplements to determine which ones are low or lacking in the Sherman-Chase diet for assaying vitamin B₁.

Results and Discussion

In this experiment for the determination of the adequacy of the Sherman-Chase vitamin B₁ deficient diet, the average weight of the rats when placed on the thiamin-free diet at 25 days of age, varied from 45 to 59 grams. The animals were depleted by litters and the period varied from 14 to 18 days for each litter, at which time their weights varied from 45 to 65 grams. The mean weight of each supplement group varied from 55.7 to 56.9 grams. In the appendix are detailed tables (Table I-VI) of the food consumption and growth records of all animals. The food was controlled within litters but there were always some animals that ate all their food and some that did not.

In Chart I, the mean gain in weight of each supplement group for the four weeks test period is shown. The rats which received only thiamin chloride, or who in addition received nicotinic acid, riboflavin, all had the same general growth curve, and their weight gains of 22.36 grams for the thiamin group, 22.42 grams for the riboflavin group, and 23.63 grams for the nicotinic acid group are close. The growth of the animals receiving crystalline vitamin B₆ and the Factor W preparation showed an increased growth that is markedly greater than the other growth curves, while those on "sulfited yeast" showed some increase. Their final gains in weight were 29.22 grams for the animals receiving vitamin B₆, 30.20 grams for those receiving Factor W preparation, and 26.15 for the "sulfite yeast" group.

Table VII summarized the mean value of the supplement groups, each of which consisted of 14 rats, one animal from each litter. There was some variation in the food consumption between the different test groups

CHART I. GAIN IN WEIGHT CURVE OF RATS ON 6 SUPPLEMENTS

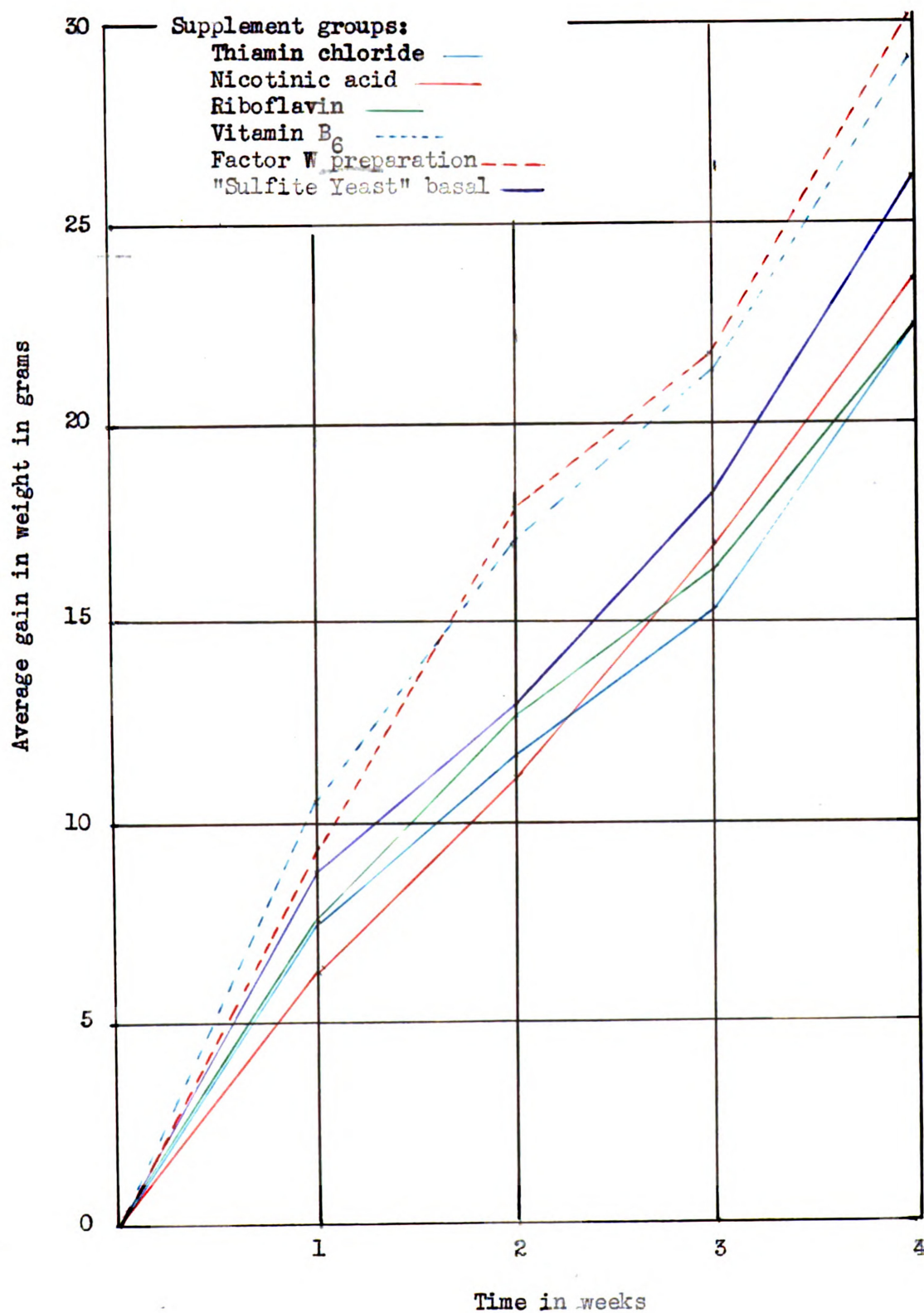


TABLE VII
SUMMARY OF DATA OF GROWTH AND FOOD CONSUMPTION OF RATS

Supplements	Number of animals	Average initial weight gms.	Average gain 4 weeks gms.	Average food consumption gms.	Gain per gram food eaten	Appetite rating* %
Thiamin chloride.....	14	56.93	22.36	116	0.1927	59
Nicotinic acid.....	14	56.79	23.64	115.1	0.2053	50
Riboflavin.....	14	55.71	22.43	115.5	0.1943	56
Vitamin B ₆	14	56.07	29.21	122.8	0.2379	31
Factor " preparation.....	14	55.79	30.21	123.9	0.2433	91
"Sulfite Yeast" basal diet....	14	55.93	26.10	120.5	0.2164	79

*Appetite rating in % represents the percentage of the time the rats in each group ate all the food offered.

in spite of the effort to control food consumption. The groups (vitamin B₆ and Factor W) which showed the greatest gain in weight had a greater average food consumption. To determine whether this increased growth was proportional to the food consumption or not; the gain in weight for each gram of food eaten was determined. From an examination of column 6 in Table I it is noticeable that those receiving Factor W and vitamin B₆ supplements had a greater gain in weight for every gram of food eaten indicating that these additions stimulated better utilization of food. The figures in the last column of Table VII represent the percentage of the time the rats in each group ate all of the food offered them. The animals receiving Factor W preparation ate all their food 91 percent of the time while those on the control diet only 59 percent of the time, indicating that the addition of the Factor W preparation stimulated the appetite. With the addition of nicotinic acid the animals did not leave any food 50 percent of the time and with riboflavin 56 percent. The addition of vitamin B₆ increased the appetite of the animals so they consumed all their food 81 percent of the time, and the substitution of sulfite yeast for autoclaved yeast in the basal diet increased their appetite by 20 percent over the control group. From this one can conclude that the addition of Factor W, vitamin B₆, or the substitution of sulfited yeast in the basal diet stimulated the animal's appetite, while the addition of riboflavin and nicotinic acid did not.

The validity of these assumptions was tested by a statistical analysis of variance of the data, which is presented in Tables VIII and IX. In Table VIII the "F" values are significantly different for the supple-

TABLE VIII

ANALYSIS OF VARIANCE OF GAIN IN WEIGHT

Source of Variance	Sum of Squares	Degree of Freedom	Variance (m.s. = $\frac{S.S.}{D.F.}$)	"F"	"t" values
Total	5,670.67	33			1. thiamin and nicotinic acid 0.59
Litter	2,713.00	13	208.69**	6.32**	2. thiamin and riboflavin 0.052
Supplements	126.73	5	25.35	5.05**	3. thiamin and vitamin B ₆ 5.17**
"Error" within animal	2,126.89	65	32.71		4. thiamin and Factor W preparation 3.65**
					5. thiamin and sulfite yeast 1.73

**Significant at 1 percent level

TABLE IX

ANALYSIS OF VARIANCE OF GAIN IN WEIGHT PER GRAM OF FOOD EATEN

Source of Variance	Sum of Squares	Degree of Freedom	Variance (m.s. = $\frac{S.S.}{D.F.}$)	"F"	"t" values
Total	273,210	33			1. thiamin and nicotinic acid 0.621
Litter	125,613	13	9,663		2. thiamin and riboflavin 0.136
Supplements	34,151	5	6,830	5.307**	3. thiamin and vitamin B ₆ 2.919*
"Error" within animal	113,446	65	1,745	3.749**	4. thiamin and Factor W preparation 3.163**
					5. thiamin and sulfite yeast 1.615

**Significant at 1 percent level

*Significant at 5 percent level

ments beyond the 1% level, indicating that the variations in growth between some of the supplements are due not to chance error but to a positive increase from some factor added to the diet. The "t" values between the thiamin control group and each of the other groups show the groups with vitamin B₆ and Factor W preparation had values that were significantly different, being greater than the 1% value for 12 degrees of freedom (N-2). Therefore, the difference in gain in weight could not be due to chance alone as only 1 animal in 100 would have gained that much by chance. The increase of the "sulfite yeast" over the control may have been due to chance errors. Thus, the addition of crystalline vitamin B₆ or Factor W preparation supplied a necessary growth stimulant lacking in the autoclaved yeast.

In Table IX the analysis of variance was determined on the gain per gram of food eaten, which eliminates the factor of differences in food consumption. The "F" values and "t" values show the same significance as in Table VIII, which indicates further that vitamin B₆ and Factor W preparation not only stimulated better growth but better utilization of food.

It is evident from these results that one can make the following conclusions:

The animals which received $\frac{1}{4}$ gamma of thiamin chloride only and those that received $\frac{1}{4}$ gamma of thiamin chloride plus 10 gamma riboflavin had the same average gain of 22.4 grams in four weeks. Thus, the addition of riboflavin had no effect on growth of the animals which seems to indicate that there is probably sufficient riboflavin present in the autoclaved yeast.

The animals receiving 1 milligram of nicotinic acid showed no increase in weight over those receiving 4 gamma thiamin chloride only, indicating that either the rats do not need nicotinic acid in their diet or that there is sufficient in the basal diet to meet their metabolic needs.

The group, whose basal diet contained sulfite treated yeast in place of autoclaved yeast showed increase in gain in weight. However the statistical analysis of the "t" value (Table VIII and IX) showed that the gain in weight was within normal limits of experimental error of the assay method. It would therefore seem that the choice between using autoclaved or sulfite treated yeast in the basal diet would depend on convenience of preparation and upon a difficulty which we encountered. All except 3 animals in this study on the basal diet using sulfite yeast developed diarrhea. One animal died as a result of the diarrhea. Even with the interference of the diarrhea, the animals gained within experimental error the same as the animals receiving the basal diet with autoclaved yeast so the yeast may have been a better source of the growth factors than was the autoclaved yeast, or the animals would have gained less.

When 10 gamma of crystalline vitamin B₆ (pyridoxine) was added there was an increased growth of 5.5 grams above those receiving only thiamin chloride supplement. This was great enough gain over the control not to be due to normal variation in growth alone. When tested statistically the "t" value was greater than the "t" value at the 1 percent level. This indicated that the vitamin B₆ is necessary for optimal growth, and that it was present in suboptimal amounts in the

autoclaved yeast. Vitamin B₆ is heat-labile and so could have been destroyed in the heating of the yeast. Some source of this vitamin would be an improvement to the Sherman-Chase diet.

The animals which received in addition to the 4 gamma thiamin chloride, 0.7 grams of Factor W preparation from liver extract each week, showed the greatest mean gain in weight of any one group on the study. This product is the substance left after the removal of the anti-anemic factor from liver extract. "The Factor W preparation does not contain vitamin B₁ but contains Factor W, pantothenic acid, and other members of the vitamin B complex necessary for growth of the rat," (private communication from Dr. Kline). The increased gain in weight of the animals receiving the Factor W preparation could then be due to the addition of Factor W and other growth factors in the preparation that were lacking or in suboptimal amounts in the autoclaved yeast. The Factor W preparation would be a good addition to the basal diet.

Therefore, the autoclaved yeast in the Sherman-Chase diet for testing vitamin B₁ is lacking or low in vitamin B₆ and some growth factor in the Factor W preparation as shown by the fact that the animals gained weight when these supplements were added to the diet. These factors should be added to the basal diet so that the test diet would be deficient in vitamin B₁ only.

The conclusions as to the necessity of Factor W and vitamin B₆ for rat growth and the dispensibility of riboflavin and nicotinic acid as supplements to the Sherman-Chase diet are in accordance with those in various reports in the literature. It was of interest however, to include in a single series of a well-controlled experiment what had

heretofore been isolated results and to see what practical application may be made for the improvement of the Sherman-Chase diet for the assay of thiamin in foods.

Summary

1. The Sherman-Chase diet for assaying food for thiamin by the rat growth method was tested to determine whether the autoclaved yeast was supplying all the vitamin B factors in amounts for optimum growth.

2. The test groups were controlled so that each group had one animal from each litter, an equal number of males and females, and the same weight at the beginning of the test period. The food consumption was controlled within litters so the average food consumption of each group was about the same. Each animal received 4 gamma of thiamin chloride each day including Sunday. The basal diet was the same for all groups except group 6 which received sulfite yeast in place of autoclaved yeast in the basal diet. The only variation was then the addition of the various vitamin B supplements (riboflavin, nicotinic acid, vitamin B₆, Factor W preparation) to determine which ones are low or lacking in the Sherman-Chase diet for assaying vitamin B₁ in foods.

3. The results indicated that with the addition of 10 gamma riboflavin daily to the diet the animals gained 22.43 grams in four weeks, while the controls gained 22.36 grams. Thus there is sufficient riboflavin in the autoclaved yeast.

4. The gain in weight of 23.64 grams of the group receiving 1 milligram of nicotinic acid was within experimental error of 22.43 grams of the control group. Therefore the diet has sufficient nicotinic acid or the rats do not need nicotinic acid for growth.

5. The substitution of sulfite treated yeast for autoclaved yeast in the basal diet gave a gain of 26.10 grams, but this gain is also within experimental error of the 22.36 grams gain of the control group.

6. The diet is low in vitamin B₆ (pyridoxine) and needs to be added to the diet as indicated by the fact that the group receiving crystalline vitamin B₆ had a gain in weight of 29.1 grams which was significantly greater than the control group as tested by analysis of variance methods.

7. The addition of Factor M preparation to the diet produced the greatest gain in weight of 30.21 grams, which was also statistically significant from the control group. Therefore for optimum nutrition the Factor M preparation is a good addition to the test diet deficient in vitamin B₁.

Bibliography

1. Chase, E. F. and H. C. Sherman, A quantitative study of the determination of the antineuritic vitamin B. J. A. C. S. vol. 53, p. 306-310 (1931).
2. Sherman, H. C., and A. Sydn, A critical investigation and an application of the Rat-growth method for the study of vitamin B. J. A. C. S. vol. 45: p. 2719-2723 (1923).
3. Goldberger, J., and R. D. Lillie, A note on an experimental pellagra-like condition in albino rats. U. S. Pub. Health Rep. vol. 41, P. 1023-1029 (1928).
4. Smith, M. I. and E. G. Hendrick, Some nutrition experiments with brewer's yeast, U. S. Pub. Health Rep. vol. 41, p. 201-207(1926).
5. Roscoe, M. H., The distribution of the vitamin B complex, I. Leafy vegetables. Biochem J. vol. 24: p. 1754-1763 (1930).
6. Sure, E., M. E. Smith and M. C. Kik, A differentiation of the so-called antipellagic factor, vitamin G. Science 73: 242-243 (1931).
7. Gyorgy, P., R. Kuhn and T. Wagner-Jauregg, Ueber das Vitamin B₂ Klin Wochenschr (1933), 12: 1241-1245 from Nat. Abst & Review vol. 2, p. 707 (1934).
8. Gyorgy, P., Investigations on the vitamin B₂ complex I. The differentiation of lactoflavin and the "rat" antipellagra factor, Biochem J. vol. 29, p. 741-759 (1935).
9. Chick, H., A. M. Copping, and C. E. Edgar, The water soluble B-vitamins IV. The components of vitamin B₂. Biochem J. vol. 29, p. 722-734 (1935).
10. Lefkowsky, S. and T. H. Jukes, The response of rats, chicks and turkeys poult to crystalline vitamin G (Flavin), J. Nutr. vol. 12, p. 515-526 (1935).
11. McHenry, E. W., and G. Gavin, The B vitamins and Fat metabolism. 1. Effect of thiamin, riboflavin, and rice polish concentrate upon body fat. J. Bio.chem. vol. 125 p. 653-660 (1933).
12. Birch, T. I. The relation between vitamin B₂ and the unsaturated fatty acid factor. J. Bio.chem. vol. 124⁹, p. 775-793 (1933).

13. Elvehjem, C. A., R. J. Madsen, F. M. Strong and D. W. Woolley, Relation of Nicotinic acid and nicotinic acid amide to canine black tongue. J. A. C. S. vol. 59, p. 1767-1768 (1937).
14. Oleson, J. J., H. C. Bird, C. A. Elvehjem, and E. E. Hart, Additional nutritional factors required by the rat. J. Bio. chem. vol. 127, p. 23-42 (1939).
15. Dann, W. J., and Y. Subbarow, Differentiation of the rat dermatitis factor and the chick dermatitis factor from nicotinic acid. J. Nutr. vol. 16, p. 183-193 (1935).
16. Birch, T. W., The requirement of the dog and rat for nicotinic acid. J. Nutr. vol. 17, p. 281-292 (1939).
17. Dimick, M. K. On the difference between thiamin deficiency in the rat and deficiencies of the other members of the vitamin B complex. J. Nutr. vol. 13, p. 607-610 (1940).
18. Lepkovsky, S., The isolation of factor I in crystalline form. J. Bio. chem. vol. 124, p. 125-128 (1933).
19. Chick, H. and A. M. Copping, The composite nature of the water soluble vitamin B III. Dietary factors in addition to the antineuritic vitamin B₁ and the antidermatitis B₂. Bio. chem. J. vol. 24, p. 1764-1773 (1930).
20. Hogan, A. G. and L. R. Richardson, Differentiation of the antidermatitis factor. Science. vol. 33, p. 17-18 (1936).
21. Docher, L. E., The concentration and properties of vitamin H. J. Bio. chem. vol. 119, n. 223-231 (1937).
22. Lepkovsky, S., Crystalline Factor I. Science. vol. 67, p. 169-170. (1933).
23. Spongy, P. and R. E. Eckhardt, Vitamin B₆ and Skin lesions in rats. Nature. vol. 144, p. 512 (1939).
24. Dimick, M. K., and C. E. Schreffler, The factor I (vitamin B₆) requirement of the rat. J. Nutr. vol. 17, 23-29 (1933).
25. Elvehjem, C. A., C. J. Koehn Jr., and J. J. Oleson, A new essential dietary factor. J. Bio. chem. vol. 115, p. 707-719 (1936).
26. Kline, C. L., C. D. Tolle, E. M. Nelson, Vitamin B₁ assay by a rat-curative procedure. J. Assoc. Off. Agr. Chem. Vol. 21, p. 305-313 (1933).
27. Williams, R. J., C. M. Lyman, G. H. Goodyear, J. H. Truesdall and D. Holaday. "Pantothenic acid" a growth determinant of universal biological occurrence. J. A. C. S. vol. 55, p. 2912-2927 (1933).

28. Williams, R. J., Pantothenic acid and its nutritional significance. *J. Nutr. supplement*, vol. 19, p. 17 (1940).
29. Hitching, G. H., and Y. Subbarow, The rat growth factors of the filtrate fraction of liver extracts. *J. Nutr.* vol. 13, p. 265-276 (1933).
30. Cleson, J. J., D. W. Woolley, and C. A. Elvehjem, Is pantothenic acid essential for the growth of rats? *Proc. Soc. Exp. Biol. Med.* vol. 42, p. 141-143 (1939).
31. Jones, T. H., Pantothenic acid and the filtrate (chick anti-dermatitis) factor. *J. A. C. S.* vol. 61, p. 975-976 (1939).
32. Williams, R. J., J. H. Truesdail, H. H. Weinstock Jr., E. Rohmann, C. M. Lyman, and C. W. McBurner, Pantothenic acid II. Its concentration and purification from liver. *J. A. C. S.* vol. 63, p. 2713-2723 (1939).
33. Williams, R. J., H. H. Weinstock, Jr., E. Rohmann, J. H. Truesdail, H. K. Mitchell and C. E. Meyer, Pantothenic acid III. Analysis and determination of constituent groups. *J. A. C. S.* vol. 61, p. 454-457 (1939).
34. Woolley, D. W., H. A. Weisman and C. A. Elvehjem, Nature and partial synthesis of the chick anti-dermatitis factor. *J. A. C. S.* vol. 61 p. 977-978 (1939).
35. Coward, K. H., J. H. Burn, H. W. Ling, B. G. E. Morgan, The determination of the antineuritic vitamin B. *Bio. chem. J.* vol. 27, p. 1719-1743 (1933).
36. Williams, R. R., R. E. Waterman, J. G. Keneztesy and E. R. Fuchman, Studies of crystalline B₁. III Cleavage of vitamin with sulfite. *J. A. C. S.* vol. 57, p. 536-537 (1935).
37. Kline, C.L., C. D. Tolle and E. M. Nelson, Basal diets for vitamin B₁ determination. *Science*. vol. 61, p. 508 (1933).
38. Dober, L. E., and E. R. Hartzler. The vitamin B₁ content of foods in terms of crystalline thiamin. *U. S. Dept. Agr. Tech. Bul.* 707 (1939).
39. Smith, H. A. and M. K. Dimmick, Stability of vitamin B complex and autoclaving. *Soc. Bio. chem. Toronto, Canada.* (1939).
40. Page, J. W., An improvement in experimental method for investigation of vitamin C. *Proc. Soc. Exp. Biol. Med.* vol. xxx (1932).
41. Steenbock, H., M. T. Sell and E. M. Nelson, Vitamin B, I.A modified technique in the use of the rat for determinations of vitamin B. *J. Bio. chem.* vol. 55, 399-410 (1933).

42. Guerrant, N. M., and R. A. Dutcher, The effect of type of carbohydrate on the synthesis of the B vitamins in the digestive tract of the rat. J. Bio. chem. vol. 112, p. 213-215 (1935).
43. Memorandum on the Second International Standard vitamin B₁; Bulletin of the Health Org. Biol. Stand., VII (no. 5) 332 (1957).
44. Hawk, P. B., E. L. Oser, A modification of the Osborne-Mendel salt mixture. Science. 74, p. 359 (1932).
45. Snedecor, G. W., Calculation and interpretation of Analysis of variance and covariance. Collegiate press, Iowa (1934).

APPENDIX

TABLE I
DATA OF GAIN IN WEIGHT AND FOOD CONSUMPTION IN CONTROL GROUP OF RATS

A. Growth

Rat number	10 [♂]	100 [♂]	174 [♀]	259 [♀]	319 [♀]	390 [♂]	459 [♀]	630 [♂]	660 [♂]	729 [♀]	789 [♀]	919 [♀]	950 [♂]	1060 [♂]	Gain of the whole group		Average Total Weight
															Total	Average	
Initial weight	61	61	65	56	58	51	58	63	57	59	50	49	53	56	797	56.93	56.93
weekly gain	1st week	14	8	7	7	12	9	0	9	7	4	4	11	7	106	7.64	64.50
	2nd week	10	-2	0	7	5	0	1	10	-1	4	9	11	-4	57	4.07	68.57
	3rd week	5	5	11	-1	4	4	4	5	6	5	0	5	-4	51	3.64	72.23
	4th week	7	7	6	9	11	1	6	11	10	8	2	8	8	99	7.07	79.30
Total Gain	36	18	24	22	27	17	20	26	24	24	15	28	11	21	313	22.36	

B. Food Consumption

																Consumption of whole group	
Rat number		1♂	10♂	17♀	25♀	31♀	39♂	45♀	63♂	66♂	72♀	78♀	91♀	95♂	106♂	Total	Average
weekly consumption	1st week	36	31	25	29	36	27	29	26	30	25	25	24	25	24	392	28.07
	2nd week	32	34	23	29	36	26	28	30	26	26	26	28	24	24	392	28.00
	3rd week	34	29	32	28	35	26	27	29	31	24	21	30	28	28	402	28.79
	4th week	41	29	33	32	35	24	29	36	32	31	25	30	33	28	438	31.36
Total food consumption		143	123	113	118	142	103	113	121	119	106	97	112	110	104	1624	116.0

TABLE II

DATA OF GAIN IN WEIGHT AND FOOD CONSUMPTION IN NICOTINIC ACID GROUP OF RATS

A. Growth

Rat number	30 [♂]	11 [♀]	20 [♂]	26 [♀]	32 [♂]	40 [♀]	50 [♂]	60 [♀]	68 [♂]	73 [♂]	79 [♀]	84 [♀]	99 [♂]	101 [♀]	Gain of the whole group		Average Total Weight
															Total	Average	
Initial weight	59	53	65	60	58	52	63	59	62	62	49	50	52	51	795	56.79	56.79
weekly gain	1st week	15	5	7	7	14	8	8	-1	4	8	8	2	3	91	6.50	63.29
	2nd week	5	-1	8	5	6	4	-5	4	10	5	4	9	6	66	4.71	68.00
	3rd week	7	3	4	3	12	8	15	00	1	9	2	7	4	80	5.71	73.71
	4th week	4	4	9	8	15	0	19	6	3	4	1	3	13	94	6.71	80.42
Total Gain	31	11	28	23	47	20	37	9	18	26	15	21	26	19	331	23.63	

B. Food Consumption

															Consumption of whole group		
Rat number		3♂	11♀	20♂	26♀	32♂	40♀	50♂	60♀	68♂	73♂	79♀	84♀	99♂	101♀	Total	Average
weekly consumption	1st week	34	27	21	30	32	27	29	25	29	28	26	24	26	21	379	27.21
	2nd week	28	34	23	29	33	25	32	30	27	27	27	28	26	24	393	28.07
	3rd week	31	26	31	27	35	26	28	26	31	28	24	30	31	24	398	28.51
	4th week	40	25	33	33	36	29	39	28	29	34	25	30	34	27	442	31.5
Total food consumption		133	112	108	119	136	107	128	109	116	117	102	112	117	96	1612	115.14

TABLE III

DATA OF GAIN IN WEIGHT AND FOOD CONSUMPTION IN RIBOFLAVIN GROUP OF RATS

A. Growth

Rat number	6♂	14♂	19♀	28♀	36♀	42♂	49♂	59♀	65♀	74♂	80♀	88♂	94♀	103♂	Gain of the whole group		Average Total Weight
															Total	Average	
Initial weight	60	57	57	55	62	45	63	54	61	57	49	56	50	54	780	55.71	55.71
1st week	14	6	2	11	9	15	4	6	4	11	10	-1	6	10	107	7.64	63.35
2nd week	5	-3	4	2	3	4	6	8	11	5	6	8	6	6	71	5.07	68.42
3rd week	7	6	0	-1	5	-3	8	0	0	9	5	10	3	2	51	3.64	72.06
4th week	2	2	9	3	10	3	4	10	6	10	-1	6	11	10	85	6.07	78.13
Total Gain	28	11	15	15	27	19	22	24	21	35	20	23	26	28	314	22.43	

B. Food Consumption

Rat number	6♂	14♂	19♀	28♀	36♀	42♂	49♂	59♀	65♀	74♂	80♀	88♂	94♀	103♂	Consumption of whole group		
															Total	Average	
1st week	34	25	20	30	30	27	26	30	31	29	26	24	27	23	382	27.5	
2nd week	27	34	23	30	36	26	30	31	28	27	28	28	26	24	398	28.43	
3rd week	34	26	24	28	32	26	28	29	33	28	24	30	31	29	402	28.86	
4th week	41	25	34	24	33	28	32	35	32	33	22	32	33	31	435	31.07	
Total food consumption	136	110	101	112	131	107	116	125	124	117	100	114	117	107	1617	115.50	

TABLE IV

DATA OF GAIN IN WEIGHT AND FOOD CONSUMPTION IN VITAMIN B₆ GROUP OF RATS

A. Growth

Rat number	50 [♂]	130 [♂]	18 [♀]	24 [♀]	33 [♀]	410 [♂]	470 [♂]	620 [♂]	690 [♂]	75 [♀]	81 [♀]	890 [♂]	93 [♀]	104 [♀]	Gain of the whole group		Average Total Weight
															Total	Average	
Initial weight	63	59	64	53	58	55	48	55	62	58	48	52	56	54	785	56.07	56.07
weekly gain	1st week	11	8	10	10	22	14	23	6	9	12	9	1	4	148	10.57	66.64
	2nd week	11	-3	9	6	2	2	5	12	7	4	9	7	7	89	6.36	73.00
	3rd week	1	11	7	3	12	-1	0	2	5	2	6	11	2	61	4.36	77.36
	4th week	4	9	5	3	6	10	17	11	3	10	3	6	12	111	7.93	85.29
Total Gain	27	25	31	22	42	25	45	31	24	28	27	33	22	27	409	29.21	

B. Food Consumption

Rat number	50 [♂]	130 [♂]	18 [♀]	24 [♀]	33 [♀]	410 [♂]	470 [♂]	620 [♂]	690 [♂]	75 [♀]	81 [♀]	890 [♂]	93 [♀]	104 [♀]	Consumption of whole group		
															Total	Average	
weekly consumption	1st week	36	31	24	33	38	28	29	28	31	29	26	23	27	24	407	29.07
	2nd week	30	34	29	31	38	26	33	32	28	27	29	28	24	24	413	29.05
	3rd week	33	34	36	26	36	25	28	29	34	27	24	33	31	30	426	30.43
	4th week	39	29	33	34	36	31	39	35	33	35	27	34	34	33	472	33.86
Total food consumption	138	128	122	124	148	110	129	124	126	118	106	118	116	111	1718	122.80	

TABLE V

DATA OF GAIN IN WEIGHT AND FOOD CONSUMPTION IN FACTOR W PREPARATION GROUP OF RATS

A. Growth

															Gain of the whole group		Average Total Weight	
Rat number	7♂	15♂	23♀	29♂	37♀	43♀	46♂	58♀	70♂	76♀	82♂	86♀	98♂	102♀	Total	Average		
Initial weight	60	58	58	58	63	49	48	51	60	50	64	50	57	55	781	55.79	55.79	
weekly gain	1st week	12	5	13	14	14	9	18	7	10	10	-2	4	13	6	133	9.50	65.29
	2nd week	12	2	12	9	11	2	7	16	5	9	12	10	1	10	118	8.42	73.71
	3rd week	2	5	11	5	5	-2	12	-2	2	2	1	8	0	2	51	3.64	77.35
	4th week	5	6	1	7	12	11	7	15	4	10	5	8	19	10	121	8.64	85.99
Total Gain	31	18	37	35	42	20	44	36	22	31	16	30	33	28	423	30.21		

B. Food Consumption

															Consumption of whole group		
Rat number	7♂	15♂	23♀	29♂	37♀	43♀	46♂	58♀	70♂	76♀	82♂	86♀	98♂	102♀	Total	Average	
weekly consumption	1st week	36	31	23	33	37	28	29	30	31	29	26	24	30	24	411	29.36
	2nd week	32	34	28	34	38	26	32	33	28	27	29	28	26	24	419	29.93
	3rd week	34	28	36	29	36	26	28	29	34	26	24	33	33	30	426	30.43
	4th week	41	29	35	34	36	31	39	35	33	33	27	36	37	33	479	34.07
Total food consumption		143	122	122	130	147	111	128	127	126	115	106	121	126	111	1735	123.93

TABLE VI

DATA OF GAIN IN WEIGHT AND FOOD CONSUMPTION IN "SULFITE YEAST" GROUP OF RATS

A. Growth

															Gain of the whole group		Average Total Weight	
Rat number	8♀	16♂	22♂	30♀	38♀	44♀	48♂	61♀	71♂	77♀	83♂	87♂	97♀	100♂	Total	Average		
Initial weight	55	62	55	59	60	48	65	52	61	49	59	50	51	57	783	55.93	55.93	
weekly gain	1st week	8	7	15	5	15	8	13	3	6	11	9	6	8	9	123	8.79	64.72
	2nd week	2	-5	6	8	5	2	4	5	9	3	6	7	-2	6	56	4.00	68.72
	3rd week	9	5	10	5	9	5	12	1	2	0	1	9	4	3	75	5.36	74.08
	4th week	7	5	7	3	10	4	9	16	5	9	4	9	9	15	112	8.00	82.08
Total Gain	26	12	38	21	39	19	38	25	22	23	20	31	19	33	366	26.14		

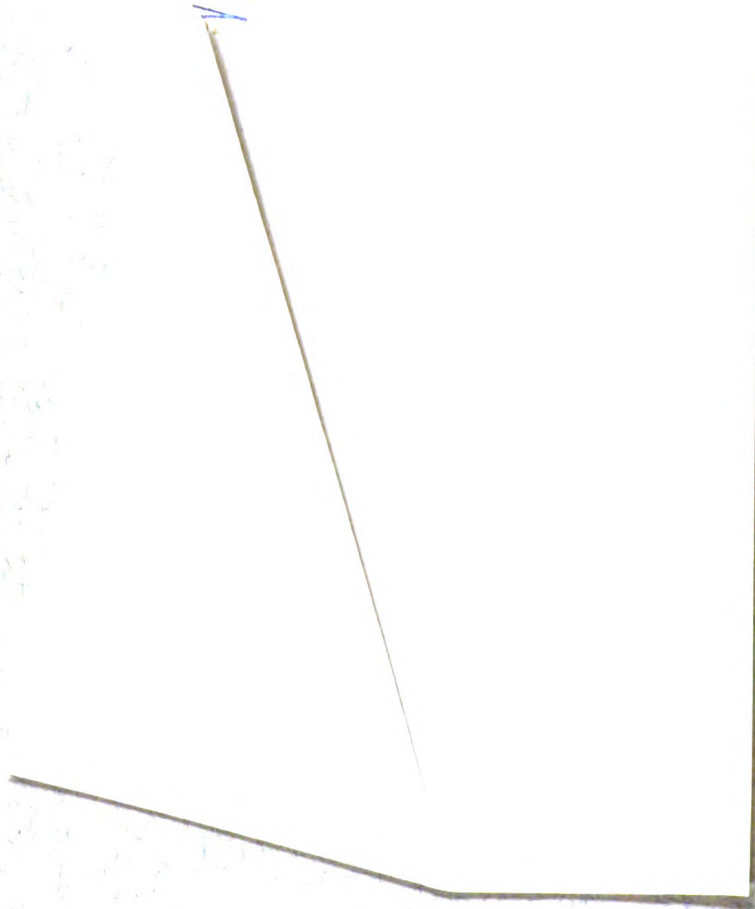
B. Food Consumption

															Consumption of whole group		
Rat number	8♀	16♂	22♂	30♀	38♀	44♀	48♂	61♀	71♂	77♀	83♂	87♂	97♀	100♂	Total	Average	
weekly consumption	1st week	29	31	23	30	38	28	29	29	31	29	26	24	26	24	397	28.36
	2nd week	30	34	28	33	37	26	34	26	28	27	29	28	24	24	408	29.14
	3rd week	33	34	36	29	36	26	28	28	32	26	24	33	32	30	427	30.43
	4th week	41	29	34	33	34	31	39	35	32	18	27	35	33	34	455	32.36
Total food consumption		133	128	121	125	145	111	130	118	123	100	106	120	115	112	1687	120.50

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