III. The Effect of Heat Treatment on the Nutritive Value of Food Yeast

The protein of food yeast (*Torula utilis*) was found to possess lower biological and growth-promoting values than the average brewers' yeast[25] used in the nutritional work of this laboratory.[26] In an attempt to find means of improving the nutritive value of such an important potential source of food protein, it was decided to submit various samples to different heat treatments. This work was undertaken in coöperation with the Pilot Plant of the Puerto Rico Industrial Development Company, as part of the studies being carried on at the School of Tropical Medicine, since 1944, on the food yeast produced there.

It has been known for some time that the nutritive value of a protein can be modified in various ways by heat treatment. In some cases it is improved;[27] in others, impaired;[28] and in still others, not affected at all.[29] The temperature, pressure, and exposure time, as well as the type of heat treatment, are factors that materially affect the nature and extent of the modification induced in the protein. Said modification may result in a higher or lower coefficient of digestibility, biological or growth-promoting values. Although the exact nature of the change suffered by the protein molecule and other substances associated with it are unknown, it has been suggested that in some cases certain amino acids seem to be protected and in others, made more liable to enzymatic reactions.

So far as the writers know, the only investigator to have studied the effect of heat on yeast proteins was Axtmayer.[30] He reported

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25. Fleischman's Type 2019.

26. J. A. Goyco and C. F. Asenjo, The net protein value of food yeast. J. Nutrition, **33**: 593–600, 1947.

27. C. O. Johns and A. J. Finks, Studies in nutrition. II. The role of cystine in nutrition as exemplified by nutrition experiments with the proteins of the Navy bean, *Phaseolus vulgaris*. J.Biol.Chem., **41**:379, 1920.

J. W. Hayward, H. Steenbock, and G. Bohnstedt, The effect of heat as used in the extraction of soybean oil upon the nutritive value of the protein of soybean meal. J.Nutrition, **11**:219–234, 1936.

28. A. F. Morgan and G. E. Kern, The effect of heat upon the biological value of meat protein. J. Nutrition, **7**:367–379, 1933.

H. A. Waisman and C. A. Elvehjem, The effect of autoclaving on the nutritive value of edestin. J.Nutrition, **16**:103–114, 1938.

29. A. Scheunert and C. Venus, Über den Nahrwert reiner FleischKost hergestellt aus rohem, gekochtem and autocklaiviertem Muskelfleisch bei Ratten. Biochem.Ztschr., Bd.219, S.186, 1932.

N. Jarussowa, Der Einfluss des Kocheus auf den Narhwert der Nahrrung. Biochem. Ztschr., Bd.207, S.395, 1929.

30. J. H. Axtmayer, Verbal communication to the College of Chemists of Puerto Rico, October 13, 1946.

that autoclaving food yeast (*Torula utilis*) at 15 pounds pressure, and at a temperature of 122° C for thirty minutes, improved its nutritive value to a considerable extent. He further observed that a mixture of rice and beans, in which the latter predominated, was effectively supplemented by heat-treated yeast.[31]

EXPERIMENTAL METHODS

Experiment 1. Autoclaving wet yeast. The yeast used in this experiment was a *Torula utilis* No. 3. After washing, and while still wet, part of it was autoclaved for a period of one hour at 20 pounds pressure and at 118° C. It was then dried by the usual process, that is, by passing the moist cell suspension through steam-heated cylinders at 130°–140° C. With the two samples of yeast (untreated and autoclaved), three different experimental diets were prepared. Their composition is shown in Table 9.

TABLE 9

Constituents	*Diets*		
	A	*B*	*C*
Autoclaved food yeasts (%)		30.0	
Untreated food yeast (%)	30.0		30.0
Cornstarch (%)	46.0	46.0	46.0
Vegetable oil (%)	10.0	10.0	10.0
Sucrose (%)	8.0	8.0	8.0
Salt mixture (%)	3.0	3.0	3.0
Cellu-flour (%)	3.0	3.0	3.0
Thiamine (mg.)	2.0	2.0	2.0
Riboflavin (mg.)	3.0	3.0	3.0
Pyridoxine (mg.)	2.5	2.5	2.5
Ca-pantothenate (mg.)	2.0	2.0	2.0
Nicotinic acid (mg.)	3.0	3.0	3.0
Inositol (mg.)	100.0	100.0	100.0
Choline (g.)	1.0	1.0	1.0
Vitamin A (USP units)	3250.0	3250.0	3250.0
Methionine (%)			0.5

For the growth experiments, twelve twenty-eight-day-old rats were used on each diet, six males and six females. The animals were kept three to a cage, males and females apart, with food and water *ad libitum*. Daily food consumption records were noted and weekly records of the body weight of each animal taken. Individual animals could be identified by an earmark.

31. J. H. Axtmayer, Tratamiento de la levadura (*Torula utilis*) para mejorar su valor nutritivo. Informe preliminar. Bol.Asoc.Méd.de Puerto Rico, 38:511–515, 1946.

TABLE 10

Growth-promoting Values of Autoclaved and Untreated Food Yeast and of Untreated Food Yeast Supplemented with Methionine

Diet	*No. of Animals*	*Average Initial Weight G.*	*Average Weight after 3 Weeks G.*	*Total Increase after 3 Weeks G.*	*Average Food Consumed in 3 Weeks G.*	*Increase in Weight per G. of Food after 3 Weeks G.*	*Increase in Weight per G. of Protein after 3 Weeks G.*	*Average Weight after 6 Weeks G.*	*Total Increase after 6 Weeks G.*	*Average Food Consumed in 6 Weeks G.*	*Increase in Weight per G. of Food after 6 Weeks G.*	*Increase in Weight per G. of Protein after 6 Weeks G.*
A	12	50.7	70.4	19.7	109.5	0.179	1.22	85.4	34.7	229.2	0.151	1.02
B	12	49.6	69.6	20.0	108.4	0.183	1.17	71.3	21.7	210.6	0.103	0.66
C	12	49.8	122.1	72.3	170.3	0.425	2.88	152.8	103.0	351.1	0.293	1.99

The feeding period lasted forty-two days. Growth response per gram of food and protein ingested was calculated on the third and sixth weeks of the experiment and is given in Table 10.

No appreciable difference was found between the values obtained for the autoclaved and the untreated yeast diets. On the other hand, the growth-promoting value of the untreated yeast diet, containing 0.5 percent of methionine, was very high so that the calculations taken on the third and sixth weeks were far superior to those obtained from the untreated yeast diet alone.

Experiment 2. Autoclaving dried yeast to be used as supplement to a typical rice and beans ration. Five hundred g. of dried yeast (*Torula utilis* No. 3) were placed in a porcelain casserole and mixed with enough distilled water to produce a souplike mixture. The casserole was placed in the autoclave for one hour at 20 pounds pressure and at 118°–122° C. Afterward, the yeast soup was poured into a porcelain tray, dried in an air oven at 40°–50° C., and finally ground to a fine powder in the Willy mill.

The polished rice and red kidney beans were prepared according to the following recipe:

Rice (polished)	270 g.	Beans (dried red kidney, left in water overnight)	113 g.
Salt	13 g.	Garlic	1 g.
Lard	45 g.	Onion	11 g.
Water	q. s.	Tomatoes	34 g.
Cook for one half hour		Salt pork	31 g.
		Green peppers	12 g.
		Parsley and other aromatic herbs	8 g.
		Salt	7 g.
		Lard	30 g.
		Annato seed	7 g.
		Water	q. s.
		Cook for two to three hours.	

After cooking, the rice and beans were mixed in a proportion of two parts of rice to one of beans, which is the approximate ratio normally served. A modified Osborne and Mendel salt mixture[32] was added to the extent of 2 percent of the dry weight of the rice and beans mixture in order to eliminate any inherent mineral deficiency in this dish. The rice, beans, and salt mixture was ground twice in a meat grinder and throughly mixed until a uniform paste was obtained.

32. P. B. Hawk and B. L. Oser, *op. cit.*

This basal diet was prepared weekly and kept in the icebox in tightly closed containers. The whole amount was divided into three equal portions for the following rations: Ration 1—basal diet only; Ration 2—basal diet plus 10 percent of its dried weight of untreated *Torula utilis* No. 3; Ration 3—basal diet plus 10 percent of its dried weight of autoclaved *Torula utilis* No. 3.

Each ration was fed to 28-day female rats *ad libitum* (three rats received Ration 1, three received 2, and four received Ration 3). Twice weekly, all the animals were fed vitamin A and B complex concentrates.[33] Rations 1, 2, and 3 were fed for four consecutive weeks. From the fifth week to the end of the experiment, the percentage of yeast in Diets 2 and 3 was increased to 20 percent of the dry weight of the rice and bean mixture. Up to the sixth week, all diets were fed in a fresh condition, but for the last two weeks, the rice and bean mixture was dried before mixing with the yeasts, and the three rations were fed in the dry state. At the end of the fourth week, two of the animals on Ration 1 received 0.1 percent of methionine, which was supplemented to the ration.

Chart III illustrates the growth curves of the animals on each of these diets, and Table 11 shows other pertinent data.

TABLE 11

Nutritive Value of Rations

Ration	*Average Initial Weight* G.	*Average Final Weight* G.	*Average Total Increase* G.	*Average Weight of Food Consumed* G.	*Increase in Weight per Gram of Food*
1	61.2	85.7	24.5	315.7	0.077
2	67.0	93.3	26.3	291.6	0.090
3	65.6	93.3	27.7	300.3	0.092

As can be seen, the autoclaved as well as the untreated yeast supplemented the rice and bean mixture only to a limited extent. Both groups of rats grew practically at the same rate, for the difference in weight gain of the two groups was very small.

Axtmayer[34] has shown that methionine supplemented rice and bean proteins very effectively. When he fed a ration of rice and

33. 6400 USP units of vitamin A; 800 ug. of thiamine; 80 ug. of riboflavin; 9.5 ug. of pyridoxine; 12.5 ug. of pantothenic acid; 2000 ug. of niacin-nacinamide; and 1.6 units filtrate factor were given weekly.

34. J. H. Axtmayer, El valor nutritivo de las proteínas. El Crisol, 1:3–8, 1947.

beans containing 0.3 percent methionine, he obtained a remarkable increase in weight. During the last four weeks of the above trial, two of the four animals on the basal rice and beans ration were fed this same ration with 0.1 percent of methionine added. The growth response brought about by this small amount of methionine was almost equal to that induced by either one of the two rations containing 20 percent of autoclaved or untreated yeast, respectively.

As a supplementary experiment, two additional groups of rats were fed Rations 2 and 3. The group receiving Ration 2 consisted of six females and eight males, while that on Ration 3 consisted of five females and six males. The animals were fed *ad libitum*, and no food records were kept.

This trial lasted for four weeks. Table 12 records the weekly weight of the two groups of animals. Considering the individual variation in experiments of this nature, it was found that the weekly gain in weight of the two groups of rats was practically the same. The results indicated that there was no appreciable difference between the supplementary action of autoclaved and untreated yeast on a rice and bean mixture.

TABLE 12

Average Weight and Total Gain, in Grams, of Rats on Rations 2 and 3
(Dried Diets with 20 Percent Yeast)

	Ration 2 (14 Animals)		*Ration 3 (11 Animals)*	
	Average Weight	*Total Increase*	*Average Weight*	*Total Increase*
Initial weight	52.0		51.5	
First week	58.9	6.9	55.9	4.4
Second week	67.5	15.5	63.8	12.3
Third week	72.8	20.8	73.1	21.6
Fourth week	78.7	26.7	76.4	24.9

Experiment 3. Supplementary action of crude and autoclaved yeast on a ration consisting of one part of cooked rice and one part of cooked red kidney beans. As no appreciable improvement was observed when food yeast was subjected to autoclaving under various conditions, it was decided to repeat Axtmayer's[35] experiment as closely as possible to see whether results similar to those reported by him could be obtained. A yeast sample (*Torula utilis* No. 3) from the Puerto Rico Industrial Development Company was utilized. This

35. J. H. Axtmayer, *op. cit.* (31).

was carefully mixed and then divided into two equal portions of 500 g. each. One portion was labeled "untreated yeast," and the other was mixed in a porcelain evaporating dish with enough water to form a souplike mixture. This mixture was autoclaved for one half hour at 15 pounds pressure and at 121°–122° C. After treatment, the yeast was poured into shallow pans, dried in an air-oven at 40°–50° C, and ground to a powder (30 mesh) in a Willy mill.

The rice was boiled in water until the grain was soft (about one half hour), then dried at 40°–50° C, and finally ground to 30 mesh. The red kidney beans were also boiled in plain water until they became soft (about three and a half hours), and then dried in an air oven at 40°–50° C. The dried beans were likewise ground in a pulverizing machine to 30 mesh.

The autoclaved and untreated yeast, as well as the cooked rice and beans, were analyzed for total nitrogen by the Kjeldahl method.[36] These ingredients were then mixed in the proportions indicated in Table 13 to obtain two rations each containing 18 percent protein (N x 6.25).

TABLE 13

Composition of Rations

Ingredients	*Percentage of Proteins by Analysis*	*Ration 1*[a]		*Ration 2*[a]	
		Percentage in Diet	*Percentage Protein from*	*Percentage in Diet*	*Percentage Protein from*
Torula utilis No. 3 (untreated)	50.93	8.84	4.5		
Torula utilis No. 3 (autoclaved)	53.37			8.44	4.5
Red kidney beans (cooked and dried)	24.25	45.36	11.0	45.36	11.0
Rice (cooked and dried)	5.60	44.65	2.5	44.65	2.5
Cornstarch		1.15		1.55	

[a] To both of these diets were added the following vitamins per kilo; 2 mg. thiamine; 2 mg. pyridoxine; 4 mg. riboflavin; 2.5 mg. calcium pantothenate; 1 g. choline. Each animal received one drop of cod liver oil per day (about 90 U.S.P. vitamin A units and 9 U.S.P. vitamin D units).

The slight differences in protein content between the autoclaved and untreated yeasts was corrected by diluting the diet with cornstarch so as to bring the percentage of protein in both diets up to the desired value of 18 percent. The higher protein content of the autoclaved yeast was no doubt due to the drying treatment, which lowered its moisture content.

36. Official and Tentative Method of Analysis. 4th ed.Assoc.Official Agric.Chemists, 1935.

To eliminate any variation in the rate of gain in weight among animals of different litters, litter-mates of twenty-eight days of age were evenly distributed between the groups receiving the two rations. Sex variation in rate of growth was also ruled out by including in each group twelve male and six female rats, that is, a total of eighteen animals per group. The animals in each of the two groups were chosen as uniform in weight as possible; the average initial weight of the animals on Rations 1 and 2 being 57.1 g. and 57.5 g., respectively.

The experiment lasted eight weeks. The two rations were fed *ad libitum*, and food consumption records were kept during the entire period of the trials. Weekly records of body weights were also taken.

Rats on Ration 2 (autoclaved yeast) grew at a slightly faster rate than those on Ration 1 (untreated yeast), as may be seen from Chart IV. However, the difference was not significant when the results were treated statistically. Table 14 records the total gain in weight made by the two groups of animals during the eight weeks as well as data on the amount of food consumed. The growth-promoting value of the bean-rice-yeast mixtures $\left(\frac{\text{weight gain in g.}}{\text{protein consumed in g.}}\right)$ was practically the same for both groups: Ration 1, 1.10; Ration 2, 1.08. This was due to the fact that, although the animals on Ration 2 (autoclaved yeast) gained more in weight (5.8 g. more), they also ate more, and consequently consumed more protein in the same period of time.

TABLE 14

Growth-promoting Values of Protein in Rations 1 and 2

Ration	*Percent Protein*	*No. of Rats*	*Average Initial Weight G.*	*Average Gain in Weight G.*	*Average Diet Consumed G.*	*Average Protein Consumed G.*	*Growth-promoting Value of Protein*
1	18	18	57.1	90.1	454.4	81.8	1.10
2	18	18	57.5	95.9	496.0	89.2	1.08

DISCUSSION

The growth data obtained indicated that there was no significant difference between the growth-promoting activity of the autoclaved and untreated *Torula utilis* proteins. The findings did not agree with those of Axtmayer,[37] who noted that autoclaved *Torula* yeast in-

37. J. H. Axtmayer, *op. cit.* (31).

duced better growth than the untreated sample, especially when fed as a supplement to a plain ration of one part cooked rice and one part cooked red kidney beans. The reason for such difference was not apparent.[38]

The growth-promoting value of the proteins in a bean-rice-yeast mixture was almost identical when autoclaved *Torula* yeast or untreated *Torula* yeast was used as a supplement.

SUMMARY

1. *Torula utilis* No. 3, autoclaved while wet for one hour at 20 pounds pressure and at 118° C. and then dried in the usual manner, was found to be practically identical in nutritive value with the yeast not subjected to heat treatment, when both were fed as the only source of protein in a diet.

2. Methionine to the amount of 0.5 percent added to the diet containing untreated yeast, as the sole source of protein, almost tripled the growth-promoting value of the protein in this ration.

3. Growth response and growth-promoting values of dried *Torula utilis* No. 3, autoclaved for one hour at 20 pounds pressure and at 118°–122° C and supplemented to a typical rice and bean ration, were found practically identical with those obtained when the untreated yeast was fed as supplement.

4. Methionine to the amount of 0.1 percent added to a typical rice and beans ration induced an increase in growth, which in four weeks' time was equal to that obtained with the yeast-supplemented ration mentioned in paragraph 3.

5. When a plain cooked rice and beans ration was supplemented with autoclaved (half hour at 15 pounds pressure and at 121°–122° C) or untreated *Torula utilis* No. 3, respectively, so as to obtain a mixture containing 18 percent protein, the growth response observed was slightly higher in the case of the autoclaved yeast than in that of the untreated sample. In an eight-weeks trial this difference was only 5.8 g. in favor of the group receiving the autoclaved yeast. Growth-promoting values of the proteins in the rations containing either autoclaved or untreated yeast were almost the same: 1.08 and

38. We have discussed our results at length with Dr. J. H. Axtmayer, who pointed out the slight differences between his procedure and ours in Experiment III:

1. That, in his paper, though the word "cooking" was used in regard to the processing of the rice and beans, they were actually autoclaved at 15 lbs. pressure for 20 minutes; that is, they were cooked under pressure.

2. That he failed to mention that a small amount of salt mixture was fed daily to all his animals.

3. That the initial weight of his animals was lower and not as uniform as that of ours.

1.10. Under the conditions of these experiments, no significant difference in the nutritive value of the autoclaved and untreated yeasts was observed.

ACKNOWLEDGMENT

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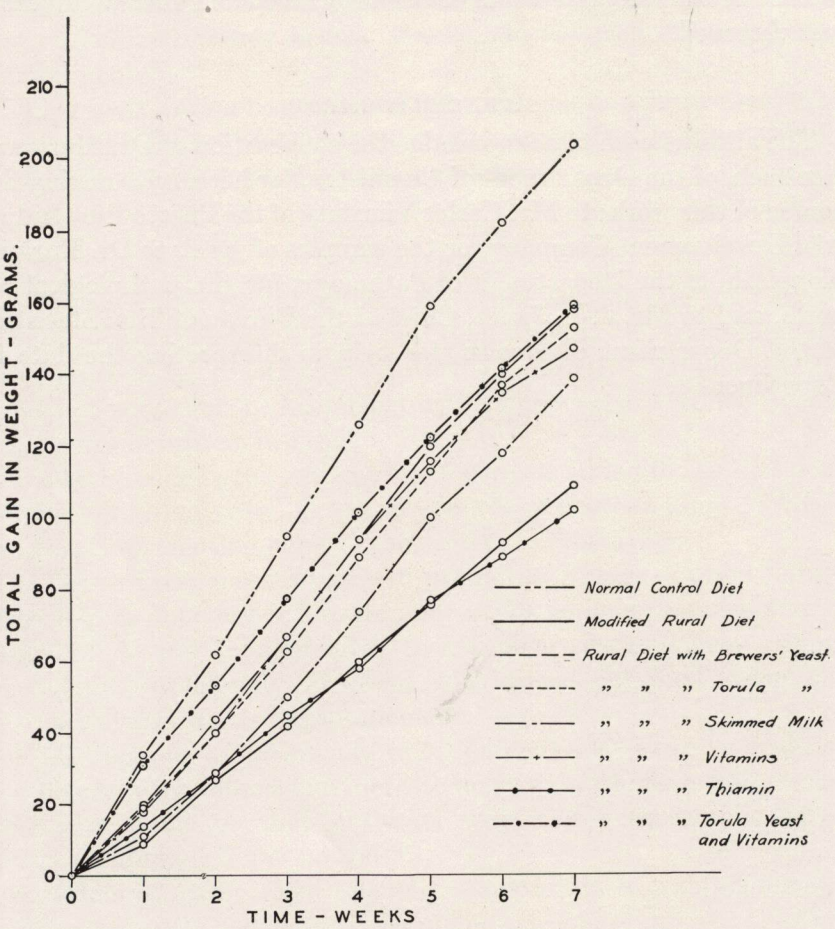

Chart I

CHART I

Growth curves of animals receiving the different experimental diets for a period of seven weeks

GRÁFICA I

Curvas de crecimiento de las ratas alimentadas con las distintas dietas experimentales durante un período de siete semanas

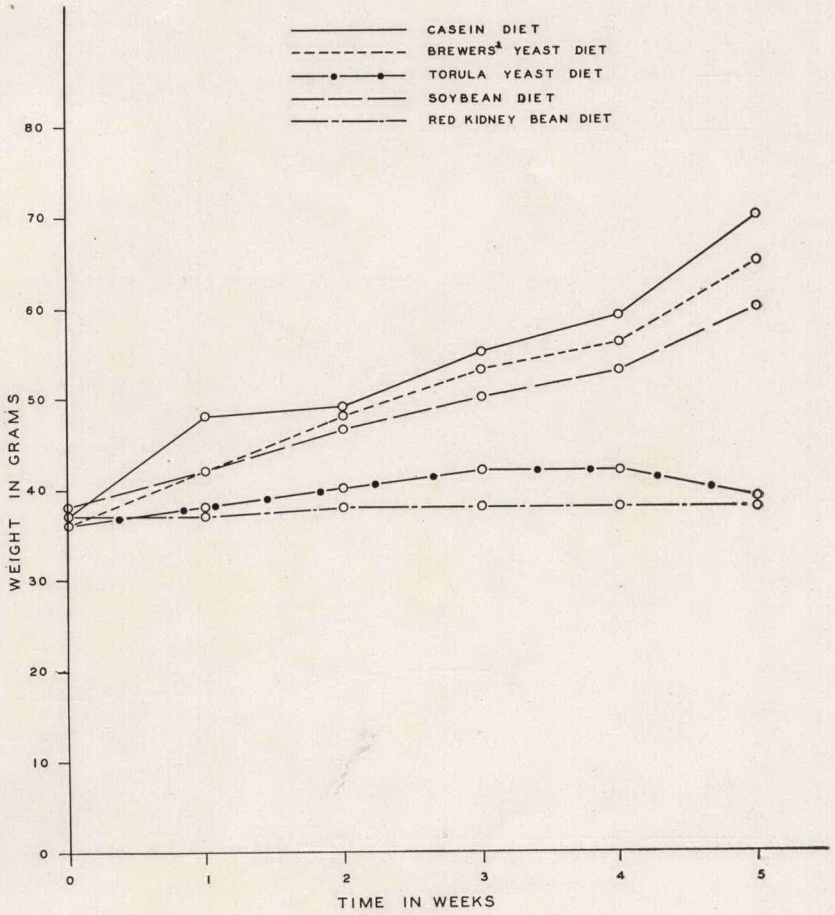

Chart II

CHART II

Pair-feeding Experiment. Growth curves of groups of rats fed diets containing 8 percent protein derived from different food sources

GRÁFICA II

Curvas de crecimiento de las ratas alimentadas en parejas con dietas compuestas de 8 por ciento de proteína procedente de distintos alimentos

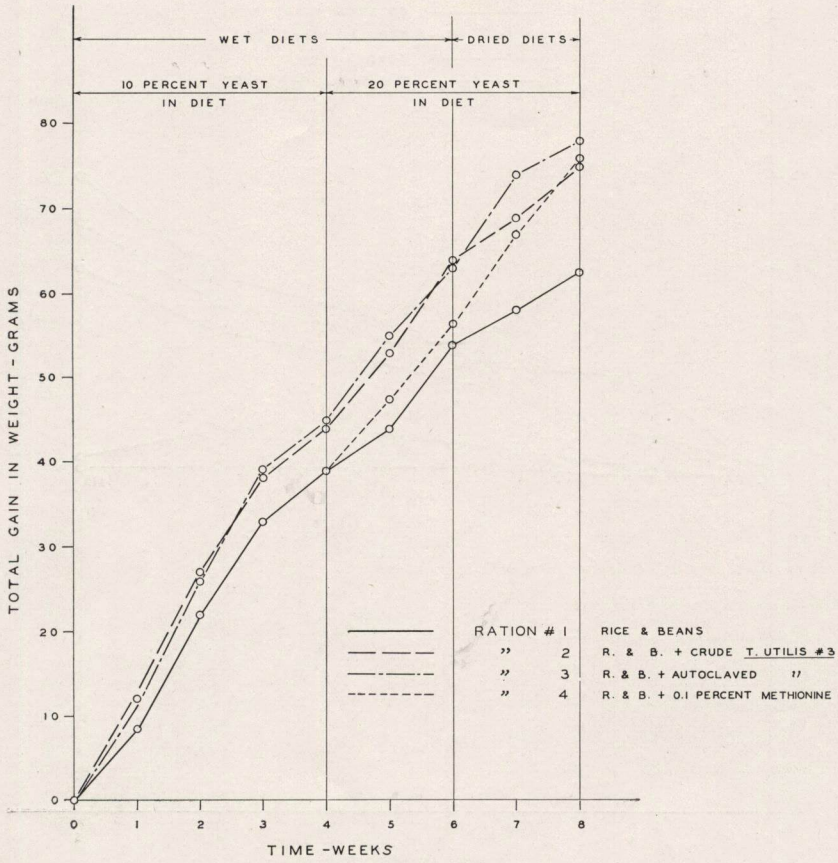

Chart III

CHART III

Growth curves of animals in Experiment 2

GRÁFICA III

Curvas de crecimiento de las ratas utilizadas en el experimento núm. 2

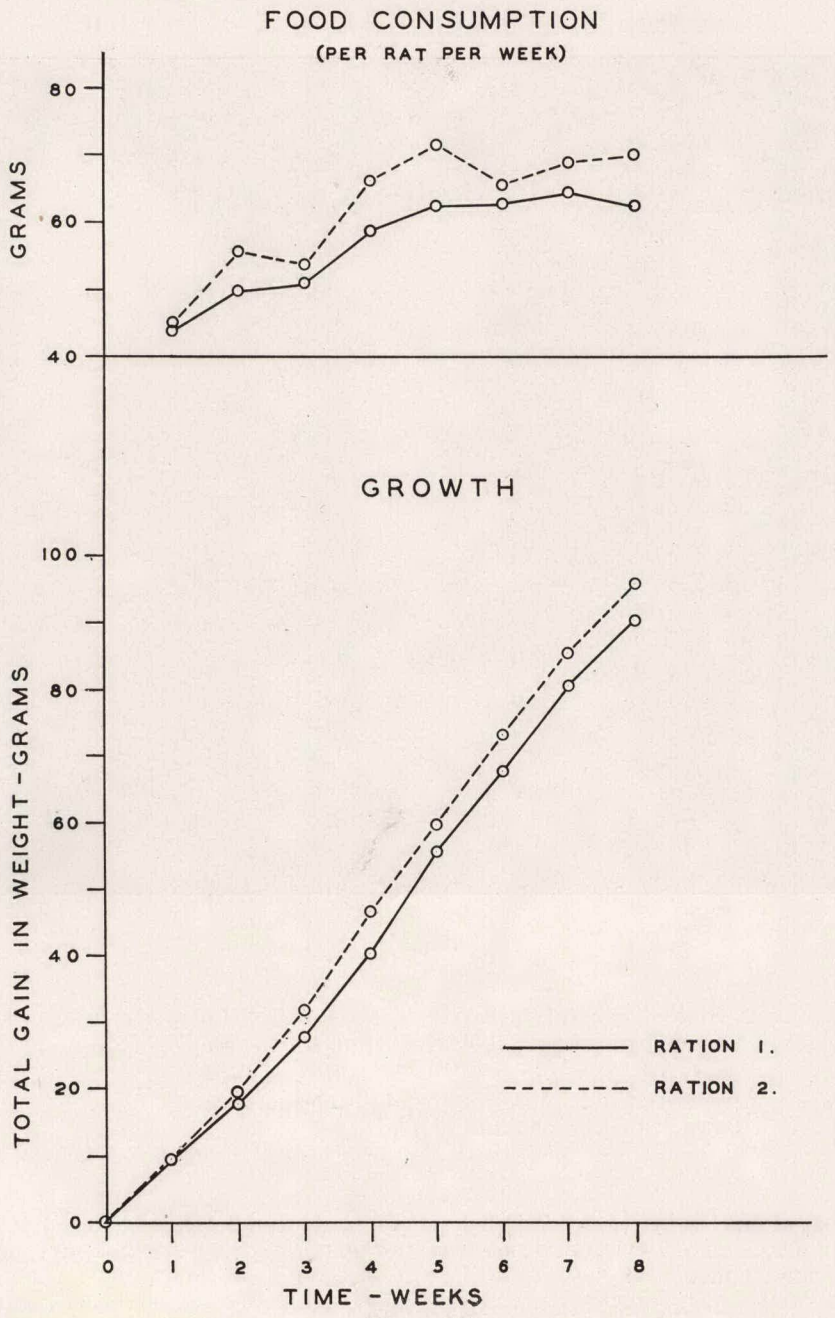

Chart IV

CHART IV

Food consumption and growth curves of animals in Experiment 3

GRÁFICA IV

Consumo de alimento y curvas de crecimiento de las ratas utilizadas en el experimento núm. 3

Fig. 1

FIGURE 1. Fourth generation rats reared on diet 3 (40 percent brewers' yeast) and receiving for a period of six weeks after weaning:

First. Diet 3 only
Second. Diet 3, plus 0.1 percent dl-methionine
Third. Stock colony diet (SB VIII)

GRABADO 1. Ratas de la cuarta generación alimentadas con la Dieta Núm. 3 (40 por ciento de levadura de cerveza) por un período de 6 semanas, después de destetadas:

1a. Con la Dieta Núm. 3 solamente
2a. Con la Dieta Núm. 3, más 0.1 por ciento de metionina
3a. Con la dieta ordinaria del laboratorio (SB VIII)