The dietary properties of yeast

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UMI
THE DIETARY PROPERTIES OF YEAST

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

BY

VICTOR G. HELLER

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V. THE VITAMINE B CONTENT OF YEAST.*

BY V. G. HELLIER.

(From the Chemical Laboratory, Iowa State College, Ames.)

(Received for publication, December 27, 1922.)

In previous communications from this laboratory by Fulmer, Nelson, and Sherwood (1) it was found that yeast can grow and reproduce for years in a medium containing only known constituents, indicating, therefore, that neither the hypothetical bios nor vitamine B need be present in the solution in order that growth proceed. These same investigators also showed that the yeast growth method, which was first employed by Williams (2), for the estimation of water-soluble B, is not only unreliable but under no circumstances can it be used for the quantitative estimation of vitamine B in our food materials, and any data obtained by this method have no significance, even though in many cases the amount of vitamine B obtained through the yeast method runs parallel to the results secured by the use of animals. Fulmer, Nelson, and Sherwood (1), furthermore, developed a synthetic medium free from all unknown factors, and this medium is the best synthetic medium that has thus far come to the attention of the author; and it is likewise the best which can be made from the constituents employed. Since yeast was supposed to contain relatively large amounts of vitamine B, and since this substance is not necessary for the growth of the yeast cell, a test of the yeast grown on the synthetic medium should reveal whether or not this organism has the capacity to synthesize this complex from simple constituents of known structure. Subsequently, Nelson, Fulmer, and Cessna (3) of this laboratory proved that yeast does

* This paper forms part of a thesis which will be submitted to the graduate faculty of the Iowa State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
synthesize vitamine B. They grew yeast in a synthetic medium; namely, medium F, inoculating this medium with yeast that had been previously repeatedly transferred, so that the amount of original constituents—either of the medium or the original yeast—was so small as to be negligible. Yeast grown upon such a medium caused a resumption of the growth of rats, which had been fed upon a diet lacking water-soluble B until all growth had ceased. Shortly after the appearance of this article Harden and Zilva (4) published some work on the capacity of yeast to synthesize vitamine B. The data were not conclusive, but some evidence was given that certain yeasts at least can synthesize this unknown dietary factor. In a recent article, MacDonald (5) comes to the conclusion that of five species of yeast which were tried all have the capacity to synthesize the water-soluble B vitamine. Recently, while this work was in the final stages of preparation, there appeared in this Journal an article on yeast as the sole source of vitamine B by Kennedy and Palmer (6). These investigators were unable to confirm the view held by many that yeast is an unusually rich source of vitamine B, and they were unable to secure normal growth and reproduction when yeast was employed as the sole source of this particular vitamine. Likewise, Evans and Bishop (7) state in a recent communication that they have not succeeded in obtaining normal reproduction in rats with yeast as the sole source of vitamine B and have postulated that perhaps another vitamine besides those already recognized may be necessary for the production of healthy young.

The object of the experiments recorded in this paper was to determine the amount of vitamine B in yeast grown upon wort and also in a synthetic medium. It would then be possible to compare the vitamine content of the two yeasts and determine whether or not the composition of the medium will influence the amount of vitamine the cell is able to synthesize. Data are also presented to show that yeast not only synthesizes the growth-promoting vitamine B, but likewise it has the capacity to produce the antineuritic vitamine.

**EXPERIMENTAL AND DISCUSSION.**

Our general procedure consisted in bringing about a suspension of growth in rats on a vitamine B deficient diet and subsequently
feeding various amounts of yeast to determine the minimum amount necessary to bring about a resumption of growth. We have, furthermore, determined the influence of various amounts of yeast on growth and reproduction. The ration fed the animals consisted of purified casein 18 per cent, salt mixture (8) 5 per cent and in other cases 3.7 per cent, filtered butter fat 5 per cent, and dextrin to 100 per cent. The casein was extracted with distilled water, acidified with acetic acid. Even though the water was changed at least once a day and often several times, nevertheless, it required as long as 4 to 5 weeks to eliminate the vitamine completely. A better, or at least a more rapid method, to remove the vitamine is to extract the casein by means of 95 per cent alcohol, using large percolators equipped with a siphon arrangement similar to a Soxhlet extractor. No casein was ever employed in these experiments which had not been previously tested in order to insure the complete absence of vitamine B, in so far as that is possible by feeding experiments.

Chart 1 shows the results obtained by adding 1.5 per cent of air-dried *Saccharomyces cerevisiae* (Fleischmann’s Race F). Up to the point marked x on the curve the animals had received a basal ration containing no vitamine B. At this point 1.5 per cent of the above yeast was added. This yeast contained no filler; in other words, it consisted of pure yeast cells. A marked response in growth resulted, but the growth was by no means normal. The female on this ration did not produce young although it was apparently from external appearances in good condition and had ample time to do so.

Chart 2 illustrates the results obtained by incorporating 2 per cent of the same yeast employed in Chart 1. The growth curves are perhaps slightly better than those obtained using 1.5 per cent of yeast. The curves approximate the normal and yet no young were produced. We have not been able to obtain young in any of our animals by the use of either 1.5 or 2 per cent of yeast in the diet.

Chart 3 shows what might be expected when the ration contains 2.5 per cent of air-dried yeast. The typical growth curves are about normal. Female 215 had a litter of four young. One of these young died in 2 weeks time and appeared in a very poor condition. The second likewise appeared to be suffering from mal-
Chart 1. Curves of growth of rats receiving 1.5 per cent of yeast (Saccharomyces cerevisiae).

Chart 2. Curves of growth of animals receiving 2 per cent of air-dried yeast (Saccharomyces cerevisiae).
CHART 3. Growth curves of rats on a synthetic diet in which the sole source of vitamin B consisted of 2.5 per cent of air-dried yeast.

CHART 4. Curves illustrating the effect of 5 per cent of air-dried yeast in the diet.
nutritional and died the following week. The third survived 4 weeks, but shortly before the time of its death looked very emaciated, and it was far below normal in weight. The fourth rat lived and grew to maturity. Its curve of growth is given in Chart 3, No. 216. The rate of growth of this animal as judged by the curve falls just below the normal. It now weighs 180 gm. and appears in a fairly good condition. During the early stages of its growth, shortly after weaning, this rat just maintained its weight for several weeks. This portion of the curve is not represented in the chart. 2.5 per cent of *Saccharomyces cerevisiae* permits of normal growth to maturity and even suffices for reproduction and the weaning and rearing of some of the young, but even though this be true reproduction is not what we would call normal.

Chart 4 gives the results obtained by using 5 per cent of air-dried *Saccharomyces cerevisiae*. The animals all grew at the normal, and better than the normal, rate. Two of the females on this ration have had young. Female 224 has produced a litter of three, and they were exceptionally fine young at birth. One died at the end of the 2nd week. It looked very well for a week or so, but later appeared in an emaciated condition, and ceased to grow. The other two were vigorous young at birth but at weaning time failed to make the gains they should. Although weaned they are now below normal weight and do not look as well as normal rats should. We have observed in our studies on yeast that the females may produce exceptionally fine young and the failure of reproduction manifests itself only at or shortly before the weaning time, when the animals either cease to grow or do so very slowly. No. 270 produced one litter at the point marked Y on the curve, but she did not take care of them. Rat 280 has produced a litter of young. The young are a little over 2 weeks old and normal in weight. This animal, however, was a mature animal when placed on the ration. 5 per cent of air-dried *Saccharomyces cerevisiae*, judged by a considerable number of experiments, allows of growth at the normal, and better than the normal, rate. It also permits of reproduction and the rearing of the young although the young do not develop as normal young should.

Charts 5, 6, and 7 show the results obtained by the use of undried yeast in place of the air-dried product. Chart 5 illustrates the fact that 3 per cent of natural undried yeast does not furnish
V. G. Heller

enough vitamine B for normal growth. This amount corresponds to 1.02 per cent of dried yeast.

Chart 6 shows the results obtained by the use of a diet containing 5 per cent of undried yeast corresponding to 1.74 per cent of air-dried solids. Growth to maturity resulted at the normal, and even better than the normal, rate. Female 232 had one litter of young, but they were destroyed. These results show the marked influence of so mild a treatment as air-drying upon the vitamine B potency of yeast. It is remarkable that this vitamine should be so stable toward heat and yet so susceptible to air-drying. The animals in Chart 2, receiving 2 per cent of air-dried yeast, did not respond as well as those in Chart 6. From numerous experiments that have been conducted in this laboratory, we are of the opinion that the undried yeast is more potent than the air-dried. But even though the animals grew to maturity at the normal rate reproduction was not normal, nor has it been in any of the animals receiving this amount of yeast.

Chart 7 shows the results obtained by the use of 6 per cent of undried yeast corresponding to 2.04 per cent of dried substance. The rats made rapid growth. Female 246 had three young but all of them died in an emaciated condition without having been weaned. The young were in very good condition and normal in size at birth. We have not succeeded in obtaining normal reproduction with this amount of yeast in any of our animals.

It is evident from these experiments that drying the yeast effects the destruction of a part of the vitamine. 2.5 per cent of air-dried yeast allows growth to maturity at the normal rate, with reproduction and rearing of a part of the young. 5 per cent of yeast permits growth at even better than the normal rate with reproduction and rearing of the young, but the young do not develop as normal young should. Experiments now in progress in our laboratory will answer the question whether it is possible to rear healthy young at all on yeast as the sole source of vitamine B, but some time must elapse before a final answer can be given. Although we may know what are the necessary dietary factors required for growth and reproduction, we do not know the concentrations of these various factors which will produce the best results. That a cell will not develop normally, without taking into consideration the concentration of the substances in which
Chart 5. Curves of growth of rats which received their sole source of vitamine B from 3 per cent of undried yeast. This amount of yeast corresponds to 1 per cent of air-dried solids.

Chart 6. Growth curves of animals receiving a diet containing 5 per cent of undried yeast equivalent to 1.74 per cent of air-dried solids.
CHART 7. Growth curves illustrating the effect of 6 per cent of undried yeast in the diet. This amount corresponds to about 2 per cent of air-dried solids.

CHART 8. Curves of growth of animals fed yeast grown on a synthetic medium.
Lot A received 2.5 per cent of this yeast in the diet.
Lot B received 3 per cent in the ration.
Lot C received 4 per cent.
it is immersed, is very well illustrated by the work of Fulmer, Nelson, and Sherwood (1) of this laboratory. Yeast is very sensitive to the concentration of the various components which comprise the medium. There is an optimum or minimum concentration for each of the constituents of the medium.

Of particular interest in this connection is the comparative amounts of vitamine B in *Saccharomyces cerevisiae* grown in wort and that grown in a synthetic medium. Considerable quantities of the latter yeast were grown in medium F, having the following composition: 100 cc. contained 0.188 gm. of ammonium chloride, 0.100 gm. of calcium chloride, 0.100 gm. of dipotassium phosphate, 0.04 gm. of precipitated calcium carbonate, 0.60 gm. of dextrin, and 10 gm. of cane-sugar. This medium must be kept at 30°C. in order to insure optimum results. The effect of adding the yeast grown on Medium F is illustrated in Chart 8. In order to save space, the results of only a few of the animals at certain definite levels are recorded. The addition of 2.5 per cent of *Saccharomyces cerevisiae* grown on a synthetic medium produced only slow growth. When 3 per cent of this yeast was incorporated in the diet, much better growth resulted; but the growth is not normal and is not as good as when 2 per cent of this type of yeast grown upon wort was used. Data not recorded here show that 3.5 per cent is not sufficient for normal growth for a period of over 3 months. When 4 per cent of this yeast is added to the diet, normal growth occurs over a period of 2 months. We have not tested the yeast on animals for a longer period, due to the extreme difficulty of obtaining a sufficient quantity. It is, therefore, evident that although yeast, which has grown upon a synthetic medium synthesizes vitamine B, it does not contain as much of this vitamine as yeast grown in wort.

**Does Yeast Synthesize the Antineuritic Vitamine?**

In a recent number of this Journal Eijkman, van Hoogenhuijze, and Derks (9) published an article in which they came to the conclusion that yeast grown upon a synthetic medium is unable to cure polyneuritis in pigeons. In view of the fact that Nelson, Fulmer, and Cessna (3) of this laboratory showed that yeast can synthesize the growth-promoting or water-soluble B vitamine Eijkman and his associates are of the opinion that what is known
as vitamine B is in reality two vitamines; the one having to do with the cure of polyneuritis, and which can, therefore, be designated the antineuritic vitamine, and the other being concerned with growth can consequently be called the growth-promoting vitamine. Eijkman, van Hoogenhuijze, and Derks cultivated \textit{Saccharomyces} isolated from baker's yeast, in a synthetic medium containing only inorganic salts and cane-sugar which they claimed was suitable for the growth of yeast, and when they fed this product to pigeons suffering from polyneuritis, it failed to establish a cure. They likewise call attention to an experiment performed previously, in which cooked, polished rice, to which Chinese rice yeast was added, failed to have any, or at least comparatively little, antineuritic potency. They noticed, furthermore, that the above species of baker's yeast when cultivated at the same temperature, namely, 27°C, in an aqueous extract of rice polishings after washing with physiological saline solution in order to remove any adhering portions of the medium, was potent in curing polyneuritis in pigeons. These same investigators likewise heated a portion of this extract of rice polishings in the autoclave at 120°C, which they claim destroys the antineuritic factor, and then grew yeast in this solution. The yeast so obtained was rich in the antineuritic vitamine while the solution which was filtered off from the yeast was found to be inactive. They also tried another strain of \textit{Saccharomyces} which was obtained from beer yeast, and used as their synthetic medium the inorganic constituents found in beer-wort. The results were the same as before. No antineuritic vitamine was formed by the yeast grown on this synthetic medium, but when grown on beer-wort it was decidedly potent.

Eijkman, van Hoogenhuijze, and Derks say in this connection: "It seems, therefore, that yeast not only takes eventually its antineuritic factor as such from the culture medium but that it is not even capable of synthesizing the vitamine unless the medium contains at least the products of decomposition of the vitamine by heating." In view of the fact that Fulmer, Nelson, and Cessna (3) of this laboratory have demonstrated that yeast can synthesize water-soluble B, a fact which appears contradictory to their work upon pigeons, they are of the opinion that vitamine B and the antineuritic vitamine are not identical.
The yeast which was used for testing the antineuritic vitamin was cultivated in the same medium employed for the growth of the yeast which was given to rats to test for the growth-promoting power. Yeast grows much more slowly upon a medium such as this than it does upon wort, and it is, therefore, a considerable task to prepare a sufficient quantity for feeding and curative experiments. Not only is it a difficult task to prepare such large quantities in a synthetic medium, but it must also be remembered that yeast so grown must be carefully guarded from contamination, for if this species of yeast did not have the capacity to synthesize vitamin B, other varieties might possess that power and, of course, such contamination would vitiate the results. The synthetic medium used by the author is by no means identical with that employed by Eijkman, van Hoogenhuijze, and Derks, and it may be that the character of the synthetic medium will influence the synthesis of water-soluble B by the cell.

The pigeons used for the production of polynearitis were full grown birds. The first bird came down with the acute form of the disease in 19 days on a polished rice diet. As expected, the appetite of this animal fell off greatly after a week or so and, in order to insure an adequate food allowance, the bird was forcefully fed. The pigeon was not fed the synthetic yeast addition until it manifested all the symptoms of polynearitis, such as the characteristic posture of the head and the total inability to use the legs. At this point the air-dried yeast, which had been grown on the medium given above, was fed by mouth and within 19 hours the animal's posture was perfectly normal except that it was very weak in the limbs. Without giving any more yeast the pigeon got up and walked in a perfectly normal manner 4 hours later. The amount of yeast used for the cure of this bird was not exactly determined, but approximately 3 gm. of air-dried yeast grown upon the synthetic medium were given.

Pigeon 2 came down with all the typical symptoms of polynearitis in 20 days. It was fed 3 gm. of moist synthetic yeast, containing approximately 1 per cent of air-dried solids. A complete cure was established in 15 hours time.

Pigeon 3 showed all the symptoms of the disease in 16 days. 2 gm. of dried synthetic yeast were then administered by mouth, and in 14 hours the bird was walking about the cage, and, although still weak, there was no question but that a cure had been effected.
Pigeon 4 had typical polynieritis at the end of 24 days. It was given 1 gm. of yeast grown upon a synthetic medium, but died during the night. Whether this amount of yeast contained too small an amount of the antineuritic vitamine, or whether the animal was in too low a physical condition, we are unable to state.

Pigeon 5 showed the disease on a synthetic ration, complete in every way except for vitamine B, in 32 days. This animal received 3 gm. of undried yeast and a cure was effected in 24 hours.

Pigeon 6 also showed typical symptoms of polynieritis in 32 days. The diet consisted of purified casein 18, salts 5, filtered butter fat 5, and dextrin to 100 per cent. 3 gm. of undried yeast grown upon a synthetic medium cured the animal in 24 hours.

The writer desires to thank Professors V. E. Nelson and E. I. Fulmer for suggesting this problem and for advice during the progress of the work.

Thanks are also due The Fleischmann Company for generously supplying us with the large quantities of pure yeast necessary for this and other research work in connection with the dietary properties of yeast.

SUMMARY.

1. The vitamine B potency of yeast (Saccharomyces cerevisiae, Race F) has been determined. 2.5 per cent of this yeast is sufficient for growth at the normal rate. Reproduction is possible at this level, but it is perhaps not normal, although young may be brought to maturity. 5 per cent of this yeast allows growth at the normal, and even better than normal, rate. Normal young have been obtained and weaned. Shortly before weaning time, the young on this ration do not develop as normal young should.

2. Drying of yeast (Saccharomyces cerevisiae, Race F) destroys some of the vitamine.

3. Saccharomyces cerevisiae grown in a synthetic medium is not as rich in vitamine B as that grown in wort.

4. Saccharomyces cerevisiae not only synthesizes the growth-promoting vitamine, but the antineuritic vitamine as well.
BIBLIOGRAPHY.

STUDIES ON YEAST.

VII. THE DIETARY PROPERTIES OF YEAST.

BY V. E. NELSON, V. G. HELLER, AND E. I. FULMER.

(From the Department of Chemistry, Iowa State College, Ames.)

(Received for publication, May 21, 1923.)

Data presented in a previous communication (1) from this laboratory showed that yeast is a comparatively rich source of vitamin B. The statement was also made that animals receiving as low as 2.5 per cent of air-dried Saccharomyces cerevisiae grew at the normal rate, and that reproduction was possible on this amount of yeast in the diet as the sole source of vitamin B, although it was perhaps not normal. Kennedy and Palmer (2) have reported experiments which lead them to believe that yeast is not an unusually rich source of vitamin B. Apparently from their experiments the efficiency of yeast depends upon the manner in which the yeast is fed. They state, furthermore, that their animals were kept under favorable conditions for reproduction, but only in a very few instances were young produced, and these were never reared.

Data are presented in this paper which demonstrate that yeast is a rich source of vitamin B, not only for growth but for reproduction as well. This is of importance because yeast is used to a considerable extent as the sole source of vitamin B in experimental rations, and it is also being widely employed in experiments having for their object the isolation of this unknown dietary factor. Evans and Bishop (3) make the statement that animals fed their synthetic diets containing yeast as the sole source of vitamin B are for the most part sterile in the first generation and wholly so in the second generation. This apparent sterility could be overcome by the incorporation in the diet of small amounts of green leaves such as lettuce. They call this unknown dietary factor necessary for reproduction vitamin X.

Data are also presented on the nutritive value of yeast proteins.
Vigorous rats from 50 to 60 gm. in weight were used. The animals received an adequate growing ration supplemented with whole milk before they were placed on the experimental diets. The rations employed in all the experiments on the vitamin B content of yeast consisted of casein 18 per cent, salt mixture 185 (4) 3.7 per cent, filtered butter fat 5 per cent, yeast in various amounts from 1 per cent up to and including 8 per cent, and the remainder of the ration to 100 per cent was composed of dextrin. The dextrin was prepared by moistening starch with 1 per cent citric acid solution and autoclaving the mixture at 20 pounds pressure for 3 hours. The casein was purified by washing for several weeks with water acidified with acetic acid. No casein was employed which had not been tested to insure the absence of all vitamin B.

The yeast employed in these experiments contained no filler, and it is known as *Saccharomyces cerevisiae* (Fleischmann’s Race E). In order to save space, the records of only a few of the many animals used will be given in the form of charts.

### Value of Various Levels of Yeast for Growth and Reproduction.

Animals receiving 1 per cent of air-dried *Saccharomyces cerevisiae* as the sole source of vitamin B as a general rule give a growth curve which is below normal. None of the females has produced young on this amount of yeast, and only one of the twelve rats employed in this experiment has grown at the normal rate for a period of 14 weeks. A few of the animals have grown normally for a period of 2 to 3 months, but after that time the curve of growth has flattened out.

When 1.5 per cent of air-dried yeast is added to the diet, the curves of growth are better than when 1 per cent is used. Several of the rats have grown at about the normal rate over a period of 4 months or more. On the other hand, a few of the animals did not grow normally for such a long period of time. Two females produced young, but the young were undersized and died shortly after birth.

When 2 per cent and higher levels of air-dried yeast are incorporated in the diet, normal growth and reproduction result. In
order to save space only two charts recording the performance of animals on two different levels of yeast intake are given. Chart 1 shows the rapid growth of first generation animals on a diet containing 2.5 per cent of air-dried yeast as the sole source of vitamin B. The same types of growth curves and reproduction records were obtained on 2 per cent of air-dried yeast and upon levels of yeast greater than 2.5 per cent. The young were normal in weight when born, and the size of the litters was practically the same as on a good breeding ration. The reproduction records of

![Chart 1](chart.png)

**Chart 1.** Curves of growth and reproduction records of animals whose diet contained 2.5 per cent of air-dried yeast as the sole source of vitamin B. There is no question of the capacity of these animals to reproduce. Only 6 of the 59 young produced on this ratio have been reared.

all the animals are shown in Table I. The data presented in this table show that of the large number of young born on the various levels of yeast intake, only comparatively few were reared. The majority of the young died during the suckling period. The sixth column in Table I headed “Total No. of young that died” includes only the young which were cared for by the mothers in a normal manner, but notwithstanding this care died in an emaciated condition. The figures in this column do not include the young devoured by the mothers nor the young which were purposely
destroyed because the mothers either died or failed to care for the young. The number of young produced by all the animals totaled 308 and of this number 53 were reared to maturity. The table also shows that on 2.5 per cent of yeast fifteen young were reared, but only six of these young were brought to maturity on this amount of yeast; the remaining nine received tomato juice equivalent to 1.5 cc. per day in addition to the yeast. It was believed that some unknown constituent was missing from the diet which is necessary for normal milk production or normal milk composition.

**TABLE I.**

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<th>Percentage of yeast in ration</th>
<th>No. of males on ration</th>
<th>No. of females on ration</th>
<th>No. of litters produced</th>
<th>Total No. of young born</th>
<th>Total No. of young that died</th>
<th>Total No. of young reared</th>
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*Signifies undried yeast containing 32 per cent of air-dried solids.

and so directly concerned in the successful nutrition of the young, and for that reason tomato juice was added. This small amount of tomato juice has been partially successful in preventing the high mortality of the young, but the same amount of orange juice does not improve the well being of the suckling.

Chart 2 shows the growth curves of second generation animals receiving 5 per cent of air-dried yeast as the only source of vitamin B in the diet. It will be noted that these animals reproduced, the resulting young constituting the third generation. The majority of the third generation young have been reared.
The young produced on these various levels of yeast (from 2 to 8 per cent of air-dried yeast and from 4 to 12 per cent of undried yeast) have been normal in every way. Repeated experiments have demonstrated the fact that when the young were placed with mothers on a good breeding ration the young were reared, whereas when the young from mothers on the breeding ration were given to the yeast-fed mothers the young succumbed. There can be no doubt that the mothers on these various yeast rations were secreting milk, otherwise it is difficult to understand how the young could live as long as 4 or 5 weeks before they died. As a general rule the young developed an emaciated condition and died in a period of time varying from 1 to 4 weeks.

The rations employed in these experiments differ from those used by Evans and Bishop (3) primarily in the fat quota. We are inclined to believe that there is an optimum concentration of fat tolerated by the animal body, and that if this optimum concentration is exceeded sterility results. We have fed four rats on a diet consisting of casein 18 per cent, filtered butter fat 5 per cent, lard 10 per cent, salt mixture 185, 3.7 per cent, air-dried

![Chart 2](https://example.com/chart2.png)
yeast 3.5 per cent, and dextrin to 100 per cent. Although the animals have grown better than the normal rate, not one of the three females, now 7 months old, has reproduced on this ration. Results similar to those obtained with yeast have been secured with wheat embryo as the sole source of vitamin B. We expect to report later on the relation of salts, fat, and wheat embryo on growth, and especially on reproduction.

It has been observed repeatedly with a large number of animals that when salt mixture 185 (4) is employed in these rations to the extent of 5 per cent, the animals are almost without exception sterile although they grow at the normal rate to maturity. Future work will determine whether the failure of the young to grow on these various levels of yeast is to be ascribed to the difference in vitamin B necessary for growth and that required for milk secretion and normal milk composition, or whether another unknown dietary factor must be taken into account. Evidence accumulated in this laboratory indicates that it is not alone a question of the level of vitamin B. Even upon 8 per cent air-dried yeast all the young were not reared, and this is four times the amount necessary for normal growth to maturity.

Value of Yeast Proteins for Growth and Reproduction.

Data in the literature are conflicting concerning the dietary properties of yeast proteins. Funk, Lyle, and McCaskey (5) state that a large part of yeast nitrogen has no food value. They state, furthermore, that young rats can live on yeast as the sole source of nitrogen for a long while, although it has not been proved that they can do so indefinitely. Osborne and Mendel (6) found that yeast proteins, at certain levels, furnish all of the amino-acids necessary for growth. Their data concerning the value of these diets for reproduction were not conclusive, however. Some of the animals proved to be sterile. In other experiments in which yeast had been used as a sole source of vitamin B they also noted that the animals were sterile, but due to the fact that two of the four males fed yeast as the sole source of protein were fertile, they drew the conclusion that it was not the yeast per se which caused the sterility.

Air-dried *Saccharomyces cerevisiae* (Fleischmann's Race F) contains 46 per cent of crude protein. The following amounts of
air-dried yeast expressed in percentage were fed in these experiments: 25, 30, 35, 40, 45, and 50. These figures correspond, respectively, to the following amounts of crude yeast protein in the diet, expressed in percentage: 11.50, 13.80, 16.10, 18.40, 20.70, and 23.00. Account must be taken of the fact that a considerable quantity of the nitrogen of yeast is present in forms other than protein, but how much of the nitrogen is so represented is not known. In order to economize space only two charts are given.

**Chart 3.** Typical curves of growth and reproduction records of animals receiving 45 per cent of yeast as the sole source of protein in the diet. This amount of yeast corresponds to 20.70 per cent of crude yeast proteins.

Chart 3 shows the type of growth curve obtained when 45 per cent of air-dried yeast is employed as the only source of protein. The ration consisted of air-dried *Saccharomyces cerevisiae* 45 per cent, corresponding to 20.70 per cent of crude yeast proteins, sodium chloride 1 per cent, calcium carbonate 1 per cent, filtered butter fat 5 per cent, and the remainder of the ration to 100 per cent was composed of dextrin. A large number of experiments performed in this laboratory demonstrate conclusively that animals are not sterile on this amount of yeast intake, and that the proteins of yeast at this level furnish all of the amino-acids necessary for growth and reproduction. Furthermore, sodium chloride
and calcium carbonate are the only inorganic constituents that it is necessary to add in order to obtain normal growth. In this respect yeast is similar to seeds which are known to be too low for normal growth and well being in the three ions, calcium, sodium, and chlorine. Animals 167, 168, 169, and 171 are young which were produced by Females 164 and 166. The curves of growth of the young are below normal. The young grew slowly for a considerable period of time shortly after they were weaned. This part of the growth curve is not shown in the chart. In spite of the fact that the curves of growth are not normal, the chart shows that two of the second generation animals have thus far had young which likewise have grown below the normal rate. Third generation animals have been reared not only on a level of 45 per cent of yeast but also on 40, 35, and 30 per cent of yeast intake. In practically all cases the second and third generation animals have grown below the normal rate, although the second generation young have almost without exception proved fertile.

Chart 4 shows typical curves of growth obtained on rations containing 30 and 25 per cent of yeast as the sole source of protein. Animals 204, 205, and 206 received the following ration: yeast
30 per cent, sodium chloride 1 per cent, calcium carbonate 1 per cent, filtered butter fat 5 per cent, and dextrin 63 per cent. At the point marked x the sodium chloride and calcium carbonate were substituted for a salt mixture equivalent to 3.0 per cent. Growth and reproduction were normal. Third generation animals have been reared on this level of yeast, although the second and third generation animals grew below the normal rate, especially during and shortly after the weaning period. Rats 289, 290, 291, 292, and 293 received a ration consisting of yeast 25 per cent, filtered butter fat 5 per cent, sodium chloride 1 per cent, calcium carbonate 1 per cent, and dextrin to 100 per cent. Growth is normal in the first generation, and the young are produced at normal intervals, but the young do not grow at the normal rate. At 11 weeks of age the surviving individuals weigh only about 50 to 60 gm. each. Whether the failure of the young to grow normally is due to an inadequacy of the ash constituents, an insufficiency of amino-acids, or to both of these factors, it is difficult to say. Animals on 50 per cent of yeast as the sole source of protein grow normally for about 3 to 4 months when the curves of growth flatten out. At this level of intake yeast is apparently toxic. Just as in the preceding experiments on yeast as a source of vitamin B, it has been conclusively demonstrated, on a large number of animals, that when these various levels of yeast are fed, together with 5 per cent of salt mixture 185 (4) the animals are almost without exception sterile.

The writers desire to thank The Fleischmann Company for supplying the yeast employed in these experiments.

SUMMARY.

1. The postulation of a new vitamin for reproduction is unnecessary. Third generation animals have been reared on 5 per cent of yeast as the sole source of vitamin B in the diet.
2. The majority of the young are not reared on synthetic diets containing yeast as the only source of vitamin B.
3. When 5 per cent of salt mixture 185 is employed in the diet, the majority of the animals are sterile.
4. Yeast proteins are good proteins. Third generation animals have been obtained on 45, 40, 35, and 30 per cent of yeast in the diet as the sole source of protein.
Dietary Properties of Yeast

5. On 25 per cent of yeast as the sole source of protein the young grow far below the normal rate.
6. There is nothing of a toxic nature in yeast to account for the failure of the young to develop when yeast is employed as the sole source of vitamin B.
7. In order to make the ash constituents of yeast complete, only the inorganic ions, calcium, sodium, and chlorine need be added.

BIBLIOGRAPHY.